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STUDIES ON THE GROWTH OF PHOMA HERBARUM VAR. MEDICAGINIS IN CULTURE [1]

Albert H. Ellingboe [2, 3]

SUMMARY

Isolates of *Phoma herbarum* var. *medicaginis* differ in growth rate during the second to fourth day after germination of single spores. This difference is particularly evident in liquid shake culture in Pfeffer's medium. Both fast- and slow-growing isolates of blackstem pathogens were isolated from alfalfa and red clover. The growth of a culture was not affected by the environment in which the spore was produced. When 2 isolates with different growth rates were grown in a common medium, one isolate did not affect the growth of the other. Chromatographic analysis of fast- and slow-growing cultures indicated that the difference is concerned with carbohydrate metabolism.

Early in a taxonomic study[4] of the organism (*Phoma herbarum* West. var. *medicaginis* Fckl.) that causes the spring blackstem disease of alfalfa and red clover, it became evident that there was considerable variation in culture growth rate, as measured by radial expansion on an agar medium, when the inoculum was a standard quantity of mycelium or a large number of spores. When the inoculum was a single spore, the growth rate was characteristic for each isolate. Schenck and Gerdemann[5] found that isolates from alfalfa grew slower than isolates from red clover.

Differences in the growth rate of single-spore cultures of the blackstem pathogen have been observed.[4, 5] The growth rate of a particular culture is not always associated with the host from which it is obtained.[4] This was one of the characters used to differentiate 2 species of Phoma—*P. herbarum* var. *medicaginis* on alfalfa and *P. trifolii* on red clover.[5] Ellingboe[4] found that each isolate had its own characteristic growth rate, and both fast and slow growers were isolated from red clover and alfalfa, though more fast growers were isolated from red clover and slow growers from alfalfa.

The object of the experiments reported herein was to determine the magnitude of the differences in growth between cultures, the time of expression of such differences, the factors that may affect growth rate, and the explanation for the differences between cultures.

MATERIALS AND METHODS.—Eight sources of cultures were used. Cultures Il-RC-1 and Il-RC-3 were isolated from red clover in Illinois, and M-RC-1 and M-RC-2 from infected red clover in Minnesota. One isolate from infected alfalfa was obtained in Illinois, Il-A5, and the other 3, M-A-1E, M-A-2, and M-A-3, were obtained in Minnesota. Single conidia from these cultures were isolated by dilution plates, and the spores were allowed to germinate 24 hours and then, with a minute quantity of agar, transferred into the plates or flasks. All cultures were grown from transfers of single spores, and the resulting culture is termed a single-spore culture.

RESULTS.—If a single spore is placed on potato-dextrose agar (PDA) in a Petri plate at 23°C, a minute colony will be produced within 24 hours. The difference between isolates resides in the growth during the next 24–72 hours. Some cultures had a distinct lag phase, producing no appreciable growth in the following 24–72 hours. Other cultures, such as Il-RC-3, had no detectable lag phase, but continued to grow without interruptions during the 24–72 hours that followed. The length of the lag phase is a characteristic of each isolate in both liquid shake culture and on PDA plates and not of spores from a certain host. Following the lag phase, all cultures appear to grow at the same rate until about the tenth day as indicated by the slope of the growth curves in Fig. 1 and 2. Replicate cultures on PDA plates may show great variance after this period, possibly because of spatial limitations and the accumulation of staling products. Cultures in liquid shake culture no longer grow as a single ball of mycelium after 10–12 days. The ball of mycelium breaks into numerous pieces, and replicate cultures commonly differ greatly. When about 100 mg (dry weight) of mycelium is produced, the cultures begin to lyse.

In the first trial it was immediately apparent that, after 4-6 days, single-spore cultures of only 1 of the 2 isolates from red clover, Il-RC-3, produced larger colonies than did single-spore cultures from the alfalfa isolates, and that the single-spore cultures from the alfalfa isolates were not identical (Fig. 1). The colony diameters of cultures of M-A-1E were inter-

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[2] Research Fellow, Department of Plant Pathology and Botany, University of Minnesota.

[3] The writer wishes to acknowledge his indebtedness to Dr. M. F. Kernkamp for suggesting this problem and assisting with the study. Acknowledgments are also due the NC-37 Technical Committee for assistance in collecting much of the material used.

[4] Ellingboe, A. H. A comparative study of the fungi causing the spring blackstem disease of alfalfa and red clover. Phytopathology 49: 764-770.

[5] Schenck, N. C., and J. W. Gerdemann. 1956. Taxonomy, pathogenicity, and host-parasite relations of Phoma trifolii and Phoma herbarum var. medicaginis. Phytopathology 46: 194-200.

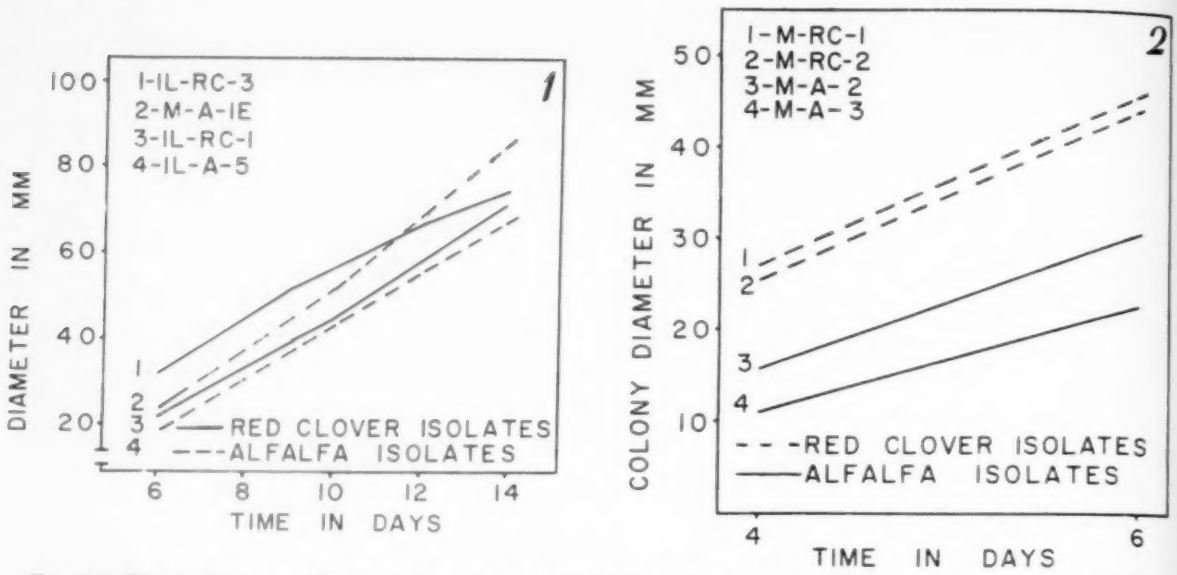


Fig. 1-2. Fig. 1. Diameter (10 replications) of colonies of *Phoma herbarum* var. *medicaginis*, in mm. obtained from single conidia grown on potato-dextrose agar in Petri plates at different intervals following subjection of the spores to conditions favorable for germination and growth. **Fig. 2.** Diameter (mm) of colonies of blackstem pathogens obtained from single conidia on potato-dextrose agar in Petri plates at 4 and 6 days following subjection of the spore to conditions favorable for germination and growth (4 sources of spores). Averages of 7 colonies for source M-A-2, of 8 for source M-A-3, of 23 for source M-RC-1, and of 16 for source M-RC-2. Conidia were obtained from pycnidia produced on leaves collected in the field and placed in moist chambers for 48 hours.

mediate between those of Il-RC-3 and Il-A-5 at 6–10 days of age. When single spores were taken directly from infected host material collected at St. Paul, the cultures from red clover sources, M-RC-1 and M-RC-2, were very similar, and their colony diameters at 4 and 6 days of age were greater than those of the cultures derived from alfalfa, M-A-2 and M-A-3. The cultures derived from the alfalfa, M-A-2 and M-A-3, were of quite different sizes on PDA (Fig. 2).

Two things were evident. First, the cultures grown from single spores obtained from a single source had almost exactly the same colony diameter if grown on PDA in Petri plates. This was not as distinct after the cultures were more than 10 days old. The second obvious conclusion is that not all cultures arising from single spores from red clover grew faster than similar cultures from alfalfa.

Growth rate in liquid shake culture.—Colony diameter on PDA in Petri plates is not always the best measure of growth, because the method fails to take into consideration the compactness or fluffiness of the colony. Therefore, single-spore cultures of 4 original cultures, Il-RC-1, Il-RC-3, Il-A-5, and M-A-1E, were grown in liquid shake cultures in Pfeffer's medium plus 1% dextrose, and growth was measured by the dry weight of the mycelium after stated periods. Ten single-spore cultures were made from each of the 4 original cultures, and 2 flasks of each original culture were harvested 6, 8, 10, 12, and 14 days after inoculation of the flasks. The single-spore cultures grew as a single ball of mycelium until about the twelfth day, after which the mycelium broke into several parts, thus increasing the number of centers of growth and also the variance between single-spore cultures from a single source culture.

The data from one experiment are presented in Fig. 3. The experiment was repeated, with similar results. The difference in growth rate was greater in liquid shake culture than on PDA. This is especially evident in the length of the lag phase in liquid shake culture. The isolate from red clover, Il-RC-3, had the shortest lag phase. On the semi-solid medium, M-A-1E grew faster than Il-A-5. However, in liquid shake culture, the difference between the 2 isolates was negligible up to 10 days following placing of the single spores in the medium, with Il-A-5 growing faster than M-A-1E after the cultures were more than 10 days old. The length of the lag phase, therefore, is partially dependent on the growing conditions.

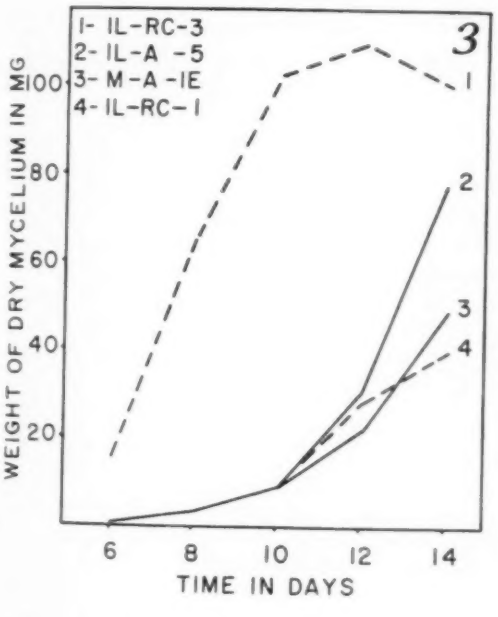
Growth following cross-inoculation.—To determine if the host from which the spores were isolated would have an effect on rate of growth from single spores, both alfalfa and red clover plants were inoculated with each of 2 isolates, 1 from each host. Single-spore cultures were obtained from M-RC-1 and M-A-3. The growth rate from a single-spore culture of RC-613 on a semi-solid medium was identical to that of M-RC-1, from which it was derived (Fig. 1). A-623 originated as a single-spore culture from isolate M-A-3, and had a growth rate identical to that of M-A-3. The 2 single-spore cultures were separately macerated in a Waring Blendor, and 1 red clover plant and 1 alfalfa plant were inoculated with each suspension of spores and mycelium. Both isolates were pathogenic on both hosts, and all plants had leaf-spot symptoms in 10–14 days. Fourteen days after inoculation, infected leaves

were removed from the plants and placed in moist chambers. Two days later, spores oozed from the pycnidia. These spores were picked from the pycnidia, serial dilutions were made, and, after 24 hours, single germinating spores were transferred into 125-ml boiling flasks containing 40 ml of Pfeffer's medium plus 1% dextrose and placed on a rotary shaker. The dried mycelial weights of these cultures are presented in Fig. 4. The respective diameters of 10-day-old colonies of RC-613 isolated from alfalfa and red clover were 64 and 62 mm. The respective diameters of 10-day-old colonies of A-623 isolated from alfalfa and from red clover were 43 and 40 mm. Differences between isolates are statistically significant; differences between hosts for each isolate are not.

The host from which the spore was isolated following cross-inoculation had no apparent effect on subsequent growth of the spore and the culture derived from that spore, on either a semi-solid or liquid medium. If the host had affected the growth of the resulting single-spore cultures, those cultures reisolated from infected alfalfa should have grown slowly and those from red clover should have grown faster. The capacity of the spore to produce a rapidly growing mycelium, therefore, must be due to the inherent capacity of the spore. The genetic constitution of the spores with respect to growth rate is expressed regardless of the environment in which the spore developed.

Growth when single spores were subjected to mass spore inoculum in the same medium.—Because growth of a single-spore culture was characteristic for each isolate, further studies were carried out to determine if rapidly growing colonies derived from single spores secrete some substance into the medium that would stimulate slow-growing single-spore cultures to grow faster. Conversely, it was desirable to see if slow-growing cultures would inhibit the growth of cultures that would ordinarily grow faster, possibly by producing some diffusible toxic substance. This was tested with the apparatus shown in Fig. 5. Cellulose casings were placed in 3 of the pockets in the flask, and a cotton plug was inserted in the fourth. A single germinated spore of RC-613 was placed in 1 casing of each of the 3 flasks. A single germinated spore of A-623 was placed in another casing in each of the 3 flasks. In the third casing, 1 flask received a mass spore inoculum of RC-613, 1 flask received a mass spore inoculum of A-623, and another flask received only sterile distilled water. In the flask proper was 500 ml of Pfeffer's solution containing 1% dextrose.

If the fast-growing culture produced some compound that the slow-growers could not and if the compounds were secreted into the medium and could pass through a cellulose membrane, a single-spore culture from a slow-growing source culture should grow and produce a much larger culture in a specified period in a medium common with fast growers than would a culture growing by itself. Conversely, if A-623 produced a toxin or a type of inhibitor, the single-spore culture RC-613 should produce less mycelium in the presence of a mass inoculum of A-623



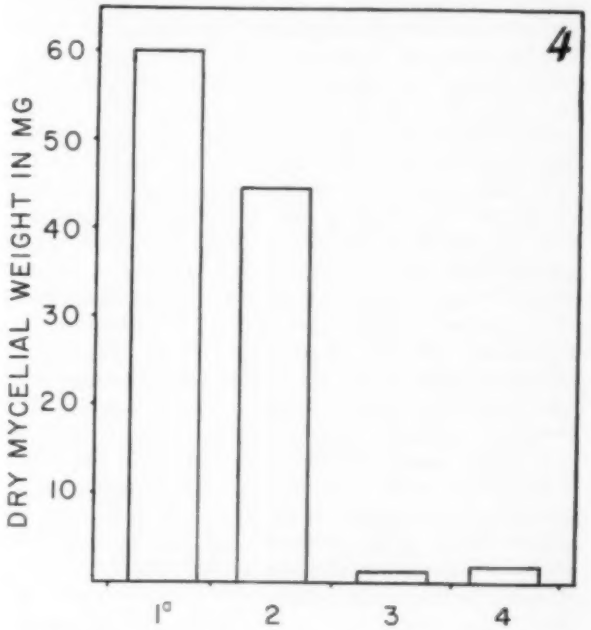


Fig. 3-4. Fig. 3. Growth expressed in mg of dry mycelium from single conidia of 4 source cultures in 40 ml of Pfeffers's medium plus 1% dextrose in 125-ml boiling flasks. **Fig. 4.** Weight of dry mycelium derived from single spores following cross-inoculation and cultures grown for 8 days in Pfeffer's medium plus 1% dextrose in shake culture. 1 = spores from alfalfa plant inoculated and infected with RC-613; 2 = spores from red clover plant inoculated and infected with RC-613; 3 = spores from alfalfa plant inoculated and infected with A-623; 4 = spores from red clover plant inoculated and infected with A-623.

Fig. 5. The apparatus used in studying cross-feeding with the growth of several cultures in a common medium separated by cellulose casings.

than in the other 2 flasks.

No cross-effect, either stimulatory or inhibitory, was observed in these experiments, as shown in Table 1. To check on the possibility that the cultures might have changed in growth rate (since the figures for dry mycelial weight in Table 1 are small), single germinating spores harvested from the same culture were also placed in 125-ml boiling flasks. The weight of dry mycelium presented as averages of 3 replicates of RC-613 was 21.8 mg and A-623 was 0.7 mg. The ratio of growth of RC-613 to A-623 follows that expected with no cross-feeding on the basis of the previous experiments, that is, the spores from culture RC-613 grew faster than spores from A-623. The explanation for the slower mycelial growth in the cellulose casing bags is probably that the bags were small and the colonies adhered to the casing rather than floating freely, as they do in a boiling flask.

Chromatographic analysis of 7-day-old cultures.—The lag phase in the growth of the slow-growing cultures is very suggestive of some type of an adaptive process, such as an adaptive enzyme. To begin an investigation on the biochemical differences between cultures, a chromatographic analysis of 7-day-old cultures was made for free amino acids and free sugars.

TABLE 1.—*Weight of dry mycelium in mg of 7-day-old cultures of black stem pathogens derived from single spores with and without association of mass spore inoculum in a common medium (Pfeffer's plus 1% dextrose)*

Mass inoculum	Single-spore source[a]	
	RC-613	A-623
Ck (sterile water)	4.4	1.2
RC-613	2.4	0.2
A-623	2.5	0.2

[a] Average of 2 replications.

Twenty 125-ml boiling flasks containing 40 ml of Pfeffer's solution plus 1% dextrose were seeded with a spore suspension, 10 with II-RC-3, and 10 with M-A-1E, and placed on a rotary shaker for 7 days. Extractions from the mycelia were made with 70% ethyl alcohol at 4°C for 24 hours, concentrated to 10 ml, and spotted on chromatographic paper. Irrigants used were collidine-lutidine-water, phenol-water, or ethyl-*n*-butanol-water.

Neither of 2 cultures studied accumulated any quantity of free amino acids. Each of the 2 isolates produced 1 faint spot on a 2-dimensional chromatogram. These 2 spots were in different positions, possibly indicating a slight difference in amino-acid metabolism, or at least in accumulation of free amino acids. When the analysis was made for free sugars, considerable differences were noticed between the 2 cultures. In the fast-growing culture, only the original carbon source, dextrose, was found in any quantity. The slow-growing culture, on the other hand, accumulated a series of free sugars. Pentoses, hexoses, disaccharides, and polysaccharides were observed when the paper was developed with $AgNO_3$. It thus appears that the difference in growth rate is probably concerned with carbohydrate metabolism. The magnitude of the difference, biochemical or genetic, has not been determined. It is conceivable, though, that the difference could be due to a single genetic locus and/or a constitutive versus an adaptive enzyme.

DISCUSSION.—The use of the character of growth rate of a culture from a single spore appears of dubious value as a taxonomic character. Though growth rate as measured by dry weight of mycelium or diameter of colony until the culture is 2–6 days old is a very stable character, it is more a characteristic of each individual source isolate rather than of isolates from alfalfa or red clover.

DEPARTMENT OF PLANT PATHOLOGY AND BOTANY
INSTITUTE OF AGRICULTURE
UNIVERSITY OF MINNESOTA
ST. PAUL 1, MINNESOTA

GREEN RING MOTTLE VIRUS AS AN ENTITY DISTINCT FROM THE SOUR CHERRY RING SPOT AND YELLOWS VIRUSES [1]

Thomas H. Barksdale

SUMMARY

The symptoms of green ring mottle on Montmorency sour cherry are similar to those of ring spot and yellows in only some respects. The apparent spread of green ring mottle was slow and continuous in an orchard surveyed annually for 7 years, and this spread was not related to the spread of ring spot and yellows. The green ring mottle virus usually occurs together with ring spot virus in nature, but is capable of being naturally transmitted in the absence of ring spot virus. It was transmitted by grafting to Montmorency and to Kwanzan trees in the absence of, and in the presence of, ring spot virus.

The English Morello variety of sour cherry is typically a symptomless host of the virus. The virus does not cause immature, pointed fruit on this variety as suggested earlier in the literature.

When introduced into the Kwanzan variety of oriental flowering cherry, green ring mottle virus causes a veinal necrosis and leaf twisting that develops in the year after fall inoculation or in the second growing season after spring inoculation. Kwanzan trees may recover almost completely in succeeding years, or they may show severe symptoms every year following inoculation.

The green ring mottle virus can exist in multiple infections in Montmorency with 1 or more of the viruses causing ring spot, yellows, line pattern, prune dwarf, and vein-clearing rosette without any marked enhancement or suppression of the symptoms caused by any of these viruses.

The green ring leaf symptom is most frequently found in nature on the basal leaves of shoots or on shoots and spurs that are growing in the inner portions of a Montmorency tree, where they are shaded. This symptom was enhanced experimentally by applying shade from budbreak until after symptoms stopped appearing. Temperatures of 70–75°F while the green ring symptom was developing favored its development. The green ring mottle virus is considered an entity distinct from the viruses of the ring spot and yellows complex.

The green ring mottle virus disease of Montmorency sour cherry (*Prunus cerasus* L.) is widely distributed in the United States and Canada but has been considered of minor importance because of the few affected trees in individual orchards. Recently, however, the disease was found to occur in a high percentage of the trees in one orchard in New York and to a lesser extent in other orchards, although more trees were affected than had been observed. This development, and the discovery that fruit borne by affected trees is typically worthless (11), led to a greater interest in the disease.

Certain similarities between the symptoms of green ring mottle and those of sour cherry ring spot and yellows suggested that the virus causing green ring mottle might be related to one of the other. In fact, "green ring yellows" has been suggested as a name for the disease (19), and the virus is listed as a strain of yellows virus in the Review of Applied Mycology (4). As found in New York State orchards, green ring mottle virus is almost invariably in double infections with the ring spot virus. Studies on symptomatology, spread, host range, and multiple infections reported herein were conducted to clarify the relationship of the green ring mottle virus to the other two.

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Based on a portion of a thesis presented in partial fulfillment of the requirements of the Ph.D. degree, Cornell University. The author thanks Dr. K. G. Parker for encouragement in these studies. Mr. Herman M. Cohn, Rochester, N. Y., made land available near Sodus, N. Y., for the field studies.

LITERATURE REVIEW.—Three symptoms are associated with green ring mottle in New York. The green ring symptom consists of yellowing or chloronemia of mature leaves, with persistent green islands and green bands forming rings. Leaves showing this symptom are eventually cast. The disease takes its name from this symptom, which was described by Rasmussen in the first publication on the disease (8, p. 61). Another leaf symptom, called "constricting chlorosis," involves 1 or more of the major veins. The leaf tissue around one of these affected veins fails to develop properly, resulting in a slight to moderate buckling of the blade, and chlorosis occurs in a strip along the vein or in 2 strips, 1 on each side of the vein. Although this symptom has been associated with the disease for several years, Gilmer et al (6) first reported it in 1957. The syndrome on the fruit, described by Parker and Klos (18), consists of a bitter flavor, of occasional patches or ring-shaped zones of necrotic tissue in the flesh, and of pits, bumps, and ring-shaped depressed lines on the surface. Photographs of the 3 symptoms have been published (6). The ring patterns in the green ring and fruit symptoms of green ring mottle are suggestive of the ring-like chlorotic mottles, etched lines, and necroses of the ring spot disease (2). The green ring symptom, with its chloronemia and leaf drop, is suggestive of sour cherry yellows (10).

No information is available on the effect of temperature and light intensity on the green ring symptom. Temperatures above 20°C have been shown to mask yellows in potted sour cherry trees (9), and a mean temperature below 60°F during the 30-day period after petal fall is associated with a severe wave of

yellows in the field (14). In contrast, warmer temperatures of 24° and 28°C are associated with severe shock symptoms of ring spot on potted trees of 5 species of *Prunus* (15).

Data on the spread of green ring mottle have not been reported, but it is thought to spread slowly (19). In New York (11) ring spot spreads slowly during the first few years after planting, and then increases rapidly; this rapid spread of ring spot is followed in a few years by a rapid spread of yellows.

Several species of *Prunus* were reported to have become infected when inoculated experimentally with green ring mottle virus (19). Until recently, however, symptoms on hosts other than Montmorency were indistinguishable from those of ring spot, and it is probable that the inoculum used in early work contained a ring spot virus contaminant. In 1955, Helton (7) described a green ring leaf symptom on Late Duke semi-sweet cherry in Idaho. In 1958, Fridlund and Diener (5) found a "reaction on Kwanzan flowering cherry" that was caused by the green ring mottle virus. These authors did not describe the reaction on Kwanzan (*P. serrulata* Lindl.), but it is presumably the one described by Milbrath (13), involving twisting and veinal necrosis of the leaf blade.

Strains of yellows virus (10, 11) and of ring spot virus (2) exist, the latter strains exhibiting interference phenomena against one another (3, 16, 17); however, there are no reports of strains of green ring mottle virus.

MATERIALS AND METHODS.—The viruses or virus complexes used in these studies were originally obtained from sour cherry trees growing in orchards. There were 3 sources of latent ring spot virus, B7/8 and 18A8 being mild selections of the etch type, and 264-2 having some necrosis along with the etch. Three sources of recurrent ring spot virus—18A4, 465-24, and A13/2—caused the etch-type symptom, and the latter also caused some necrosis. The source A10/1 contained the etch-type recurrent ring spot virus, the yellows virus, and the plum line pattern virus. The C20/1 source contained a latent ring spot virus of the chlorotic mottle type, yellows virus, and prune dwarf virus. Another source of yellows virus, 37M7, caused severe dwarfing and contained latent ring spot and prune dwarf viruses. HFC 16/7 contained a severe form of the vein-clearing rosette virus as well as latent ring spot and yellows viruses. The source of green ring mottle virus was 99A5, and 2 sources (Bndl 3/24, 99A3) contained both green ring mottle and ring spot viruses. All of the sources listed above were from Montmorency, but there was also a source (EM23/27) from the English Morello variety of sour cherry that had green ring mottle and latent ring spot viruses. A source of healthy buds, C21/1, came from a virus-free selection of Montmorency. In some instances, orchard trees were used directly as sources of inoculum; however, the sources used most were maintained by propagation or by inoculation of healthy young trees in experimental plots near Sodus, N. Y. The designations given the sources are those of K. G. Parker.

Inoculations were made by either "T"-budding or chip budding, and the buds were held in place by rubber strips. Each bud shield contained both xylem and phloem.

Indexing for ring spot virus was done with the Shirofugen cherry, as described by Milbrath (13). A double-budding method was sometimes used as well for indexing for ring spot and/or other viruses. This method involved putting both a diseased and a healthy bud on a healthy rootstock simultaneously, the rootstock being cut back to force growth of the healthy "indicator" bud.

SYMPTOMATOLOGY.—The green ring symptom can often be found on a few leaves of infected trees from mid-June to early September. For diagnostic purposes, however, it is used most easily during the few weeks from late June to mid-July, when a wave of the symptom occurs. This wave may occur concurrently with the wave of sour cherry yellows, but may be up to 3 weeks later.

Shoots that are shaded or weak will bear a greater number of affected leaves than will vigorous shoots exposed to sunlight much of the day. A 5-year-old tree, inoculated with both green ring mottle and ring spot viruses 3 years earlier, was selected for a study of this matter at the height of a wave of the green ring symptom. The tree was divided into thirds, from the ground up, and the leaves on the lower and middle thirds were placed in 2 groups, depending on whether the shoots that bore them were growing on the outside or the inside of the tree. Of the leaves showing the green ring symptom, 14% were on the lowest outside portion of the tree, 14% on the middle outside portion, 30% on the lowest inside portion, 42% on the middle inside portion, and none on the top third.

A study was also made of the effect of the application of shade to 2 groups of 4 trees each. The trees in both groups had been systemically infected with the green ring mottle and ring spot viruses for at least 2 years preceding the test. Within each group there were 2 pairs of trees inoculated from a different source, and shade was applied to cover 1 member of each pair. The shade was produced by a 5×10-ft. "roof" of jungle cloth supported about 6 ft. from the ground. This allowed sunlight to reach the trees for a few hours in the early morning and late afternoon, but they were completely shaded for most of the day.

On a bright sunny day, the light intensity under the cloth varied from 400 ft-c at a height of 1 ft. above the ground to 125 ft-c 4 ft. above the ground. At the respective levels for the unshaded trees, it varied from 500 to over 1000 ft-c. The temperature under the jungle cloth roof was no different from that at the same heights in the interior of unshaded trees.

Shade was placed over 2 trees in the first group at the beginning of June, removed for 2 weeks from June 23 to July 5, and then replaced until the beginning of August. The severity of the green ring symptom was no different on these intermittently shaded trees than on the unshaded trees. In the second group,

however, 2 trees remained shaded from budbreak until the beginning of August. On these 2 trees, the green ring symptom was much more severe than on the unshaded trees (Table 1). On trees that did not receive shade, the leaves that developed the green ring symptom were in the center of the trees, and thus in at least partial shade during much of the day. The constricting chlorosis symptom was not markedly affected by shading. No fruit could be observed, because of a late spring frost.

A study of the effect of temperature on the development of the green ring symptom was made with 1-year-old potted Montmorency trees. The trees were inoculated with both green ring mottle and ring spot viruses and were then placed in a coldroom at 40°F. Following a 3-month dormancy period, they were placed in 1 of 3 chambers (4.5×2.5×3.5 ft. high, inside dimensions) where the temperature was controlled within ±2°F and the relative humidity fluctuated between 60 and 100% a number of times each day. Each chamber contained a bank of 10 30-watt white fluorescent lights and 5 15-watt incandescent bulbs. This provided a light intensity of 600 ft-c 1 ft. below the bank of lights. Throughout the experiment, the light period was 14 hours and the dark period 10 hours.

All 3 chambers were maintained at 55°F at the beginning of the test. Over a period of 6 weeks the temperatures were gradually raised, more rapidly in the second and third chambers than in the first, until chamber no. 1 was 65°F in the day and 55°F at night, chamber no. 2 was 70° and 55°F, and chamber no. 3 was 75° and 60°F. The chambers were then left at these settings for a month.

Symptoms had begun to appear by the end of the first 6 weeks, first on trees in the warmest chamber, no. 3. These trees had more growth than trees in the other chambers. During the entire test, only 13 of the 48 inoculated trees developed symptoms. This low percentage was consistent with the results of field studies, in which autumn-inoculated trees usually do not develop symptoms until the second growing season after inoculation.

On each tree the wave of symptoms lasted about 10 days, and then ceased except for sporadic appearances. By the end of the month during which temperatures remained fixed, symptoms had appeared on 2 trees in the coolest chamber (no. 1), on 3 trees in chamber no. 2, and on 5 trees in chamber no. 3. At this time the temperatures in the second chamber were left unchanged, whereas those in the third, or warmest, were dropped to 60°F in the day and 55°F at night, and those in the first, or coolest, were raised to 75° and 70°F. Within 2–10 days, 5 plants, in chamber no. 1 developed the green ring symptom; 3 had never shown symptoms before, and 2 had become arrested in symptom development at least 2 weeks before the temperature increase. Although the green ring symptom appeared at all temperatures tested, it was most abundant under the highest temperature.

TABLE 1.—*Development of green ring leaf symptoms on unshaded 4-year-old Montmorency trees and on comparable trees shaded from budbreak, the trees having been systemically infected by the green ring mottle and ring spot viruses for 2 years*

Tree no.	Treatment	No. leaves showing green ring symptoms on date indicated[a] 7/10/58	7/14/58	7/23/58	Total
1	None	0	3	0	3
2	Shaded	28	6	23	57
3	Shaded	27	8	40	75
4a	Shaded branch	5	4	13	22
4b	Most of tree not shaded	1	5	4	10

[a] Symptomatic leaves were removed after each count.

During the temperature studies, 3 shoots on each tree were tagged and a record was kept of the position of each leaf showing the green ring symptom. A tally of the number of affected leaves showed 19 in the basal position, and then 12, 7, 5, 3, 2, 1, and 1 in a decreasing progression toward the tips of shoots.

SPREAD.—An orchard in western New York was surveyed annually for 7 years for virus disease spread.[2] Of the 378 trees in this orchard, 80 had shown symptoms of green ring mottle by 1958; of these, 44 had all 3 symptoms, 22 had constricting chlorosis and the fruit symptom, 2 had the fruit and green ring symptoms, 1 had constricting chlorosis and green rings, 9 had the fruit symptom only, 1 had constricting chlorosis only, and 1 had green rings only.

The comparative rates of spread for green ring mottle, ring spot, and yellows are given in Table 2. The peak years for ring spot expression were 1952 and 1953. From experience in other orchards, the writer assumes that many trees had become infected with latent ring spot virus before initiation of the survey. The amount of yellows was negligible in 1952 and 1953, after which it began increasing rapidly.

TABLE 2.—*The comparative rates of spread of ring spot, yellows, and green ring mottle in a Montmorency sour cherry orchard*[a]

Year	No. of newly observed infections: Ring spot	Yellows	Green ring mottle[b]
1952	64	7	38
1953	68	2	24
1954	9	0	11
1955	12	31	1
1956	11	1	3
1957	2	7	2
1958	6	51	1

[a] There were 378 trees in the orchard.

[b] Includes trees showing the green ring, constricting chlorosis, or fruit symptoms of this disease, or any combination of these symptoms.

[2] This survey was initiated in 1952 and continued in 1953 by K. G. Parker and E. J. Klos. Beginning with 1954 it has been conducted by the writer with the help of others, including G. Schmid and W. R. Allen.

Unfortunately, the surveys in 1954, 1956, and 1957 were not conducted at a time favorable for the observation of yellows.

From an examination of the case histories of diseased trees in this orchard, it is often difficult to tell which disease appeared first on any one tree. It is clear, however, that in multiple infections involving green ring mottle and ring spot, ring spot appeared first in 7 instances and green ring mottle first in 1. Of the 22 multiple infections involving yellows, yellows appeared after green ring mottle in 16 cases, whereas it appeared either first or simultaneously in the remaining 6.

With respect to the spread of green ring mottle virus, some of the 24 and 11 new infections respectively recorded in 1953 and 1954, probably occurred earlier but had been missed in earlier surveys. This seems likely since there was wide variation in the intensity of symptom expression from year to year on individual trees, and infected trees were not symptomatic in some years. The writer considers the 1–3 new infections recorded each year from 1955 through 1958 as typical and representative of a slow, steady rate of spread of green ring mottle.

Evidence of slow spread of green ring mottle has been obtained in other orchards, and a particularly striking example was seen in 1956. In a solid block of 702 Montmorency trees that had been virus-free when planted in the fall of 1950, 2 trees on Mazzard (*P. avium* seedlings) root and 1 on Mahaleb (*P. mahaleb* L.) became diseased. These 3 trees were widely scattered in the orchard and were removed. No green ring mottle has been observed before or after 1956.

Host range.—One of the problems in working with the green ring mottle virus has already been alluded to in the literature review: the difficulty of finding a culture of the green ring mottle virus free of contamination with the ring spot virus. Such a culture was found in 1 tree in the orchard mentioned in the last section.

In 1953, budwood from this tree was used to inoculate 3 healthy Montmorency trees and for propagation. This material was free of ring spot virus as indexed on Montmorency and Shirofugen flowering cherry. Of the 3 Montmorency trees inoculated, 1 tree developed symptoms of green ring mottle on the inoculated leader after 3 years; the next year the entire tree showed symptoms.

Of the 8 budlings grown from the original orchard source, only 3 were later shown, by inoculations to Montmorency and Kwanzan cherry, to contain the green ring mottle virus. Since 1953 was the first year in which symptoms of green ring mottle were apparent in the orchard tree, it is likely that the virus had not yet become evenly distributed throughout the tree and that some of the budwood used for propagation had actually been virus free.

The use of budwood from these 8 budlings when they were young, at a time when it was presumed that they all contained the green ring mottle virus, led to a number of "inoculations" of plants in the host-range and multiple-infection studies with what was later found to be virus-free buds. Most of the work reported in this and the next section was done with a combination of the green ring mottle and ring spot viruses.

Montmorency.—In 1956, when it was known which of the 8 buildings were infected, an attempt was made to transmit green ring mottle virus (99A5) in the absence of ring spot virus to healthy Montmorency trees in the greenhouse. One group of trees had been propagated from a healthy source and another from a source containing latent ring spot virus (B7/8). Some trees in each group were left unbudded to serve as checks. Inoculations were made as the trees were breaking dormancy, and no symptoms were seen on them in the ensuing period of growth. After a dormant period, they were again returned to the greenhouse. Four of 6 inoculated trees in the group containing ring spot virus expressed symptoms, and 1 of 5 trees in the group that was healthy when inoculated expressed symptoms. Of the unbudded checks, 1 tree in the group containing ring spot virus and 3 trees in the healthy group remained symptomless; the remaining checks did not survive into the second growing season. A third group of trees, which had been propagated from a source (99A3) containing both green ring mottle and ring spot viruses, was carried along in this experiment and was not inoculated. Only 3 of 7 trees in this third group expressed symptoms of green ring mottle. The failure of symptoms to appear on 4 of these trees may explain the failure to observe higher percentages of transmission in the other 2 groups.

Oriental flowering cherry.—Several sources whose virus content had been determined by K. G. Parker and E. J. Klos were selected for inoculation studies involving the Shirofugen and Kwanzan varieties of oriental flowering cherry (*P. serrulata* Lindl.). Table 3 presents a composite record of several experiments performed between 1953 and 1957. Any sources containing ring spot virus, as shown by prior inoculations to sweet and sour cherry or to peach, caused a local necrotic reaction on Shirofugen. Among viruses commonly found in New York sour cherry orchards, the green ring mottle virus was the only virus causing the systemic reaction of veinal necrosis and leaf twisting on Kwanzan. The viruses causing line pattern, prune dwarf, yellows, and ring spot are not involved in the development.

An interesting reaction is recorded in Table 3: Two of the sources used for autumn inoculation caused severe symptoms on Kwanzan the first year after inoculation, but there was recovery in the following years. A third source caused a severe reaction during all 3 years. This may indicate the existence of strains of green ring mottle virus.

One member of each pair of Kwanzans that had been inoculated with B7/8, A10/1, and 37M7 (cf. Table 3) and that had remained symptomless for 1 or 2 years was inoculated in the fall of 1955 with green ring mottle virus (99A5). During the next growing season,

only the member of each pair that had been inoculated developed the twisted leaf symptom. The other member of each pair remained symptomless. Both members of each pair indexed positive for ring spot virus on Shirofugen, as was to be expected.

An attempt was made to recover the virus from Kwanzan trees showing the twisted leaf symptom. Healthy 1-year-old potted Montmorency trees in a dormant condition were brought into the greenhouse and inoculated as the leaves were unfolding. Nine trees were inoculated with buds from a Kwanzan tree that had been inoculated previously with ring spot, yellows, and green ring mottle viruses (37M7 plus 99A5). Seven trees were inoculated with buds from a Kwanzan tree containing ring spot and green ring mottle viruses (EM23/27). Four trees were left unbudded to serve as checks. Leaf symptoms were observed during 2 growing seasons in the greenhouse, but the trees could not be kept for observation of fruit symptoms. Constricting chlorosis and/or green ring symptoms developed on 8 of 9 Montmorency trees in the first set, and on 3 of 7 trees in the second.

Sweet cherry.—Several years before the author began these studies, K. G. Parker had inoculated the Schmidt and Napoleon varieties of sweet cherry (*P. avium* L.) with 2 virus sources (EM23/27 and Bndl 3/24), both of which contained green ring mottle and ring spot viruses. The symptoms that appeared on leaves of these trees in following years were typical of those induced by infection by the ring spot virus alone. The trees were observed for several years after they had reached bearing age, and their fruit was thought not to vary in any way from that of uninoculated healthy trees. The writer took buds from these sweet cherry trees and used them to inoculate healthy Montmorency trees by the double-budding technique, with Mazzard seedlings as the intermediate host. During the second season after inoculation, symptoms of green ring mottle appearing on Montmorency showed that 6 sweet cherry trees were infected with green ring mottle virus, i.e., 1 Napoleon and 2 Schmidt trees were infected as a result of inoculation with each of the 2 sources.

In the spring of 1955, the writer inoculated healthy pairs of 6 sweet cherry varieties (Windsor, Black Tartarian, Schmidt, Lambert, Napoleon, and Emperor Francis) with buds containing both green ring mottle and ring spot viruses (99A3) or with ring spot virus alone (B7/8). A pair of trees in each variety was left uninoculated. A group of Montmorency trees was inoculated from each bud source to serve as checks on the virus content of the inocula. The trees were examined for leaf symptoms through 3 growing seasons, but the sweet cherry trees did not bear fruit until the fourth season.

Reduction in growth was striking in the first growing season after inoculation of the trees with either the virus combination or ring spot virus alone; the inoculated trees had about half as much terminal growth as did the uninoculated checks. In any year, the sweet cherry trees inoculated with both viruses developed no foliage symptoms that differed in any way from the symptoms on trees inoculated only with the ring spot virus.

All varieties but Schmidt bore fruit during the

TABLE 3.—*Symptoms caused by stone fruit viruses on Kwanzan and Shirofugen oriental cherries*

Viruses[a] used and time of inoculation	Symptoms[b] on Shirofugen	Symptoms[c] on Kwanzan in stated year after inoculation		
		First	Second	Third
Autumn inoculations:[d, e]				
Latent ring spot (B7/8)	+	0	0	0
Latent ring spot, yellows, and prune dwarf (C20/1)	+	0	0	0
Latent ring spot, green ring mottle (Bndl 3/24)	+	tw2,db3	tw2	tw0.5
Healthy buds	—	0	0	0
Recurrent ring spot, yellows, line pattern (A10/1)	+	0	0	0
Latent ring spot, yellows, prune dwarf (37M7)	+	0	0	0
Latent ring spot, green ring mottle (99A3)	+	tw2,db1	tw0.5	tw0.5
Latent ring spot, green ring mottle (EM23/27)	+	tw2,db2	tw3	tw3
No buds	—	0	0	0
Spring inoculation:				
Latent ring spot (B7/8)	+	0	0	
Latent ring spot (264-2)	+	0	0	
Recurrent ring spot (18A4)	+	0	0	
Latent ring spot, green ring mottle (99A3)	+	tw0.5	tw1	
Green ring mottle (99A5)[f]	—	0	tw1	
No buds	—	0	0	

[a] Virus source designations are those of K. G. Parker; for more detailed description, see Materials and Methods.

[b] A local necrotic reaction around the inserted bud is indicated by +; — indicates that the inserted buds lived and a healthy callous formed around them.

[c] A systemic type of disease reaction in which the leaf veins become necrotic and the leaves twist downward is indicated by "tw"; db = die back; symptom severity is rated 0.5, 1, 2, or 3 for trace, mild, moderate, or severe, respectively.

[d] Autumn inoculations are averaged for 2 trees; for spring inoculations, observations for the B7/8, 264-2, and 18A4 sources are averaged for 4 trees, those for 99A3 and "no buds" are averaged for 2 trees, and those for 99A5 are for 1 tree.

[e] Inoculations from the first 4 sources listed under autumn inoculations were made by K. G. Parker.

[f] After showing the twisted leaf symptom, this Kwanzan tree indexed free of ring spot virus on Shirofugen.

fourth growing season after inoculation. Within any variety, the color, shape, and stage of maturity of the fruit on trees of any given inoculation history were no different from those on the uninoculated trees. Twelve fruits from each tree were selected at random and sliced in several places for examination of the flesh. In no variety did the combination of green ring spot viruses cause any flesh necrosis, as it does in the Montmorency cherry.

English Morello sour cherry.—Parker and Klos (18) reported that they had transmitted green ring mottle virus to Montmorency from an English Morello having small, pointed, late-maturing fruit. This symptom was known to occur in several English Morello orchards, but annual surveys had failed to demonstrate spread of the disorder. In the present study 10 trees showing this fruit symptom were selected from 2 orchards, and 5 other trees not showing this symptom were selected from another orchard. Healthy Montmorency budlings were inoculated in August with buds from the 15 trees, and several were inoculated with buds from a source known to have both ring spot and green ring mottle viruses. Any given source was used to inoculate 3 Montmorency trees. Over the 3-year period following these inoculations, symptoms of green ring mottle appeared only on those inoculated from the check source and from the English Morello tree that Parker and Klos had already shown to be infected.

In another test, healthy English Morello trees were inoculated with Montmorency buds from 3 sources containing the green ring mottle plus ring spot viruses and from 2 sources containing green ring mottle, ring spot, and yellows viruses. Six trees were inoculated from each source, and 6 were left unbudded to serve as checks. During the 3 growing seasons after inoculation, the only symptoms developed were those of ring spot or of ring spot and yellows. None of the English Morello trees bore immature, pointed fruit at harvest time.

MULTIPLE INFECTION.—Data from only 1 of several multiple infection tests are presented in Table 4. For this test, healthy Montmorency trees were propagated on Mazzard stock, and buds containing both green ring mottle and ring spot viruses were used for the propagation of trees on both Mazzard and Mahaleb roots. About 75% of the diseased budlings on both stocks developed green ring mottle symptoms while in the nursery row. When the trees were 2 years old they were inoculated in the autumn with 3 buds from

TABLE 4.—*Symptoms on Montmorency trees propagated from healthy buds or from buds containing both the green ring mottle and ring spot viruses and subsequently inoculated with various stone fruit viruses*

Host tree rootstock and virus content	Inoculum bud virus content[a]	Symptom ratings on shoots indicated[b]		
		On shoots from inoculum, 1957	On shoots of host tree[c]	
			1957	1958
Mahaleb; green ring mottle and ring spot	Latent ring spot (B7/8)	0	g1,c1,ft	ct
	Latent ring spot (18A8)	0	g2,c2,ft	ct
	Recurrent ring spot (A13/2)	RSt	gt,ct,RS2	RSt
	Recurrent ring spot, yellows, line pattern (A10/1)	RSt,ct	gt,c1,RSt,Y2	RSt,ct
	Latent ring spot, yellows (C20/1)	0	Y3	ct
	Vein-clearing rosette, ring spot, yellows (HFC16/7)	0	Y2,VCR2	VCR2
	Healthy	0	gt,ct	ct
Mazzard; healthy	Latent ring spot (B7/8)	0	RSt	0
	Latent ring spot (264-2)	0	RSt	0
	Latent ring spot (18A8)	0	RS2	0
	Recurrent ring spot (465-24)	0	RS1	0
	Recurrent ring spot (A13/2)	RSt	RS3	RSt
	Recurrent ring spot, yellows, line pattern (A10/1)	RSt	RS1	RSt
	Latent ring spot, yellows (C20/1)	0	Y2	0
	Latent ring spot, yellows, vein-clearing rosette (HFC16/7)	0	Y1,RS1,VCRt	VCR2
	Healthy	0	0	0
Mazzard; green ring mottle and ring spot	Latent ring spot (B7/8)	ct	g2,c2,ft	ct
	Latent ring spot (264-2)	0	g2,c1	cl
	Latent ring spot (18A8)	0	g2,c1,ft	ct
	Recurrent ring spot (465-24)	RSt	g1,c1	ct
	Recurrent ring spot (A13/2)	RSt,ct	g2,c1,ft	RSt,ct
	Recurrent ring spot, yellows, line pattern (A10/1)	RSt	g2,c3	RSt,ct
	Latent ring spot, yellows (C20/1)	0	c1	cl
	Latent ring spot, yellows, vein-clearing rosette (HFC16/7)	0	RSt,Yt	VCR2
	Healthy	0	g2,c1,ft	ct

[a] Inoculations made in late summer, 1956.

[b] RS = ring spot; Y = yellows; VCR = vein-clearing rosette; g = green ring leaf symptom; c = constricting chlorosis; f = fruit symptom; t,1,2,3 = trace, mild, moderate, and severe disease reactions, respectively.

[c] Ratings averaged for 3 trees in both Mazzard lots and for 4 trees in the Mahaleb lot.

one of several virus sources (cf. Table 4). In the following spring, the trees were pruned in order to force growth of at least 1 of the inoculum buds.

Green ring mottle virus passed into the shoots grown from the inoculum buds of 1 latent and 2 recurrent sources of ring spot virus without affecting the severity of ring spot symptoms as compared with corresponding shoots growing on the trees propagated from a healthy bud source. The addition of various viruses to 2 lots of trees already infected with green ring mottle and latent ring spot viruses did not cause symptoms of green ring mottle to be any more or less severe than on the trees of each lot that received healthy buds.

A strain (HFC16/7) of the virus causing vein-clearing rosette (6) was included because of the considerable attention this disease has attracted recently in sour cherry plantings in the northeast. The disease has also been called simply "rosette" (1, 12). The strain used is a severe one causing narrow thickened leaves with sharply serrated margins; it appears to mask leaf symptoms caused by other viruses that may be with it in a multiple infection.

Discussion.—Although similarities between the symptoms of green ring mottle and those of yellows and ring spot do exist, the data presented constitute evidence that the causal agent of green ring mottle is distinct from the viruses causing ring spot and yellows.

First, the symptoms of green ring mottle on Montmorency or on oriental flowering cherry are recognizable and distinct from those of ring spot and yellows. Summer inoculation with ring spot virus causes local gumming and necrosis around the bud inserted on Shirofugen branches; inoculation with the green ring mottle virus does not. On Kwanzan, autumn inoculation with the ring spot and yellows viruses produces, in the following summer, nothing more than an occasional mild chlorotic mottle on a few leaves; inoculation with green ring mottle virus causes a severe veinal necrosis and downward twisting of the leaves.

Second, the spread of green ring mottle in orchards is slow and follows no distinct pattern with regard to the spread of ring spot or yellows. The fact that green ring mottle is almost always found in nature in trees that have ring spot virus as well is not necessarily an indication of an association between the two; instead, this is likely due to the abundance of ring spot. If the natural spread is almost wholly dependent on ring spot virus accompanying it or being already present in a host tree, it is not clear why green ring mottle has not increased more than it has in orchards that have a high percentage of the trees affected by ring spot. In these studies, the virus causing green ring mottle was observed to spread naturally, both with and without ring spot virus. It was also transmitted by grafting to Montmorency and to Kwanzan cherry, both in the absence and in the presence of ring spot virus.

Third, no evidence was observed of interference between green ring mottle and either ring spot or yellows viruses. In the studies with multiple infections, as in the orchard survey data, there is ample evidence that the green ring mottle virus exists in double infections with the ring spot virus or in multiple infections with the ring spot and yellows viruses. Green ring mottle virus was never replaced by ring spot or yellows viruses nor were symptoms of recurrent ring spot and yellows masked by green ring mottle. On some trees in which yellows virus was a part of the complex, only yellows was seen in some years. This could be expected, however, in a year in which yellows symptoms were severe, since yellows develops before the green ring leaf symptom, and on the very leaves that are most likely to show the green ring leaf symptom.

Fourth, the diseases differ with respect to temperature relations and to shading effect. Yellows is most severe when a long cool period follows petal fall. The data presented here indicate that the green ring symptom is most severe when warm temperatures prevail during its development. The green ring symptom develops best on shaded shoots, whereas yellows will develop on shoots all over the tree and on many that are in the tree tops, exposed to direct sunlight during much of the day.

Department of Plant Pathology
Cornell University
Ithaca, N. Y.

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THE INHIBITORY ACTIVITY OF PLANT JUICES ON THE INFECTIVITY OF POTATO VIRUS X[1]

W. Blaszczak,[2] A. Frank Ross, and R. H. Larson

SUMMARY

Juices from 26 species or varieties of plants inhibited the infectivity of the brown spot strain of potato virus X (PVX). The infectivity of PVX was wholly suppressed by juices from *Pelargonium hortorum*, *Chenopodium album*, *C. amaranticolor*, and 2 varieties of *Capsicum frutescens*. Inhibition was very distinct with juices of the following: *Datura stramonium*, *D. metel*, *Solanum integrifolium*, *S. tuberosum*, *Spinacia oleacera*, *Phaseolus vulgaris*, *Trifolium pratense*, and *Vicia faba*.

Tenfold dilution of juices with distilled water in most cases removed the inhibition or decreased it remarkably, except for some species like *P. hortorum*, *Chenopodium amaranticolor*, and *Capsicum frutescens*. Dilution revealed the presence of augmenters of PVX infectivity in juices of *Nicotiana debneyi*, *N. glutinosa*, *Lycopersicon esculentum*, *Cucumis sativus*, and *Gomphrena globosa*. Heating juice 10 minutes at 60°C removed inhibition in many cases but was generally less efficient than dilution. Juice of *P. hortorum* did not lose its inhibitory activity when heated 10 minutes at 100°C. When the juice of *P. hortorum* was sprayed on assay plants, inhibition was still complete when the leaves were inoculated 20 days later; the juices of *C. album*, *S. oleacera*, and *Capsicum frutescens* sprayed on assay leaves were still inhibitory when inoculation was delayed 6–7 days, the longest period tested. The concentration of inhibitory agent was higher in young top leaves than in older ones or in the stem. Juices of many species retained their full inhibitory action after storage for several weeks at about 4°C.

The phenomenon of virus inhibitors in juices from different plant species has been studied by several workers (4, 5, 6, 7, 8, 12, 13, 18). Duggar and Armstrong (8) found that juice of Pelargonium and of *Datura stramonium* L. inhibited tobacco mosaic virus (TMV). Bawden and Kleczkowski (4) reported that juice from strawberry, from leaves as well as fruits, was highly inhibitory to TMV. Kuntz and Walker (12) found that juice from spinach was inhibitory to several viruses. Diachun (7) reported that juice of *Phytolacca decandra* L. decreased virus infectivity less when applied on Phytolacca than when applied on other plant species. Crowley (6) proved the presence of plant virus inhibitors in seed of cucumbers, and McKeen (15) studied inhibition by juice of *Capsicum frutescens* L. Fungi and bacteria have also been found to produce virus inhibitors (2, 3, 15, 16). Some of the inhibitory substances in plant juices have been isolated, and some investigations have been made of removal of the inhibition by different treatment of juices, such as dilution, heating, and use of a buffer (4, 5, 12, 17, 19). No reports, however, compare the effects of juices from many plant species on virus infectivity.

In the present study, the inhibitory activity of juices from plant species commonly used in virus research was investigated. In addition, some of the properties of the inhibitors were determined, and methods of removing the inhibitors were sought.

Materials and methods.—The brown spot strain (10, 13, 14) of potato virus X (PVX) was used throughout this investigation. Infected leaves of Turkish tobacco (*Nicotiana tabacum* L.) were ground in a meat grinder, and the juice was squeezed through a double layer of cheesecloth. The juice was placed in several test tubes, frozen immediately, and stored at −10°C. A single batch of juice so prepared served

[1] Accepted for publication May 19, 1959.
[2] Adjunct, Phytopathological Institute, College of Agriculture, Poznan, Poland; on leave during 1958-59 on a Fellowship of the Rockefeller Foundation.

as inoculum throughout the study. When inoculum was needed, a tube of juice was thawed just before use; hereinafter, this juice (or a 1:15 dilution) is referred to as "stock inoculum."

Juices to be tested for inhibitors were extracted in the above manner from 2- to 3-month-old greenhouse-grown plants; unless otherwise stated, the juices were not frozen. Juices from supposedly healthy plants were tested for PVX, and only those found free of this virus were used.

Except where otherwise stated, the assay plant was *Gomphrena globosa* L., with each plant trimmed to 4 pairs of leaves. Since the number of local lesions produced on this plant varies with leaf position (16), each inoculum was applied at the same leaf position the same number of times. The gauze-pad method of inoculation was used; before inoculation, the leaves were lightly dusted with 400-mesh Carborundum. The leaves were not rinsed after inoculation nor were they wet during subsequent plant waterings. All plants were kept in a greenhouse at about 21°C, and lesion counts were made 10 days after inoculation.

The 4 treatments in the preliminary basic test were 1) stock inoculum diluted 1:10 with distilled water, 2) stock inoculum diluted 1:10 with the test juice, 3) stock inoculum diluted 1:10 with the test juice previously diluted 1:10 with distilled water, and 4) stock inoculum diluted 1:10 with the test juice previously heated 10 minutes at 60°C. All dilutions were made just before inoculation. Inhibition was measured by the difference between the number of lesions induced by a test inoculum and the number induced by the control (treatment 1, above).

Juices that showed strong inhibition of PVX in the basic test were further studied—in tests with different dilutions of healthy-plant juices (with and without comparable dilution of the stock inoculum), juices heated at various temperatures, juices sprayed on test leaves before inoculation, and juices from different plant parts. In addition, tests were made with different dilutions of juices from PVX-infected plants, other assay hosts, and TMV. Each experiment was repeated at least once when 12 replications were used and at least twice when 8 replications were used.

RESULTS.—*Inhibition of PVX by juices from several plant species.*—In the basic tests, juices from each of the 26 healthy plant species or varieties used inhibited PVX infectivity (Table 1). Juices of *Capsicum frutescens* (both varieties), *Chenopodium album*, *C. amaranticolor*, and *Pelargonium hortorum* totally sup-

TABLE 1.—*Lesions induced on Gomphrena globosa by a stock inoculum of potato virus X diluted with distilled water (control) or with crude juices, diluted juices, or heated juices of various plant species*

Source of diluent	Lesion numbers induced by control[a,b]	Lesion induction,[b] as percentage of control		
		Juice not diluted	Juice diluted 1:10	Juice heated 10 min. at 60°C
Capsicum frutescens L. var. California Wonder	14.5	0	12	0
C. frutescens L. var. Wisconsin Lakes	26.0	0	2	0
Datura metel L. (P.I. 234199)[c]	36.4	3	60	7
D. stramonium L.	38.0	5	68	18
Nicotiana debneyi Domin	31.0	66	142	107
N. glutinosa L.	51.1	57	145	72
N. rustica L.	19.4	45	121	103
N. tabacum L. var. Samsun	19.8	72	130	129
N. tabacum L. (Holmes' NN)[d]	58.9	32	100	79
N. tabacum L. (Turkish)	10.0	54	118	101
Nicandra physaloides Gaertn.	15.9	16	82	51
Lycopersicon esculentum Mill. var. Bonny Best	16.5	19	155	107
Petunia hybrida Vilm. var. Comanche	22.8	34	115	101
Physalis floridana Rydb.	26.5	30	135	105
Solanum integrifolium Poir.	17.6	9	67	28
S. tuberosum L. (USDA Seedling 41956)	67.8	9	83	33
Chenopodium album L.[e]	19.6	0	26	1
C. amaranticolor Coste et Reyn.	12.0	0	3	0
Spinacia oleacera L. var. Bloomsdale	10.1	8	88	18
Phaseolus vulgaris L. var. Bountiful	27.2	9	108	50
Trifolium pratense L.	31.5	8	45	
Vicia faba L.	17.7	8	86	69
Vigna sinensis Endl. var. Black Eye	34.2	22	109	103
Cucumis sativus L. var. A and C	17.5	38	141	106
Gomphrena globosa L.[f]	3.7	38	243	73
Pelargonium hortorum Bailey	25.5	0	0	0

[a] Distilled water used as diluent. Each figure represents the mean number per leaf.
[b] Each inoculum (including control) was applied to 2 leaves on each of 12 plants.
[c] P.I. refers to the accession number of the Plant Introduction Section, U.S.D.A.
[d] Containing the *N* gene from *N. glutinosa* (11).
[e] Juice from frozen plants that were old.
[f] Tested on *N. rustica*.

pressed the infectivity of PVX, whereas juices of *Datura metel, D. stramonium, Solanum tuberosum, S. integrifolium, Spinacia oleacera,* and 3 leguminous species caused about 90% inhibition. Juices of species of the genus *Nicotiana* in general inhibited PVX to a smaller degree.

In most cases, diluting juices 1:10 with distilled water removed the inhibitory action partially or completely. Only juices from *P. hortorum, C. amaranticolor,* and *C. frutescens* were strongly inhibitory after dilution. In contrast, diluting some juices not only removed the inhibitory action but markedly increased PVX infectivity, e.g. 55% with juice of tomato and 143% with juice of *G. globosa.*

Heating juices 10 minutes at 60° was generally less efficient than dilution in removing the inhibitory activity. In some cases, however, heating removed the inhibitory action partially or completely, but it had no effect on the inhibitory activity of juices from *C. frutescens, C. album, C. amaranticolor,* or *P. hortorum.*

In 2 other experiments, juices from 6 varieties of potato were tested. Mean numbers of lesions induced by the controls were 33.9 per leaf in one test and 38.8 in the other. When juices of the varieties tested were used as diluent for PVX, the relative numbers of lesions, as percentage of the control, were 24.1% for Sebago, 21.5% for USDA seedling 41956, 18.2% for Saco, 17.0% for seedling 1335, 12.1% for Tawa, and 6.9% for Cherokee. The juices of the first 3 varieties were obtained from the leaves and top parts of stems of young plants.

Influence of dilution on inhibitory activity of juice.—Juice of *Chenopodium album* was obtained from plants approaching maturity. Leaves of *C. album* and *P. hortorum* were frozen before the juices were obtained. Juice from *Capsicum frutescens* was obtained from fresh, unfrozen leaves. Stock inoculum was diluted 1:10 with various dilutions of each juice and assayed on *G. globosa.*

The inhibitor in juice of *P. hortorum* appears to be very powerful, since its action was still apparent at dilutions of 1:2000 (Fig. 1-A). The inhibitory activity of the juices of *Chenopodium album* and *Capsicum frutescens,* respectively, disappeared at dilutions of 1:100 and 1:500. In fact, PVX infectivity was augmented slightly at these and slightly higher dilutions. Since Chenopodium juice appeared more inhibitory at 1:2000 than at slightly lower dilutions, it could be that the inhibitor was still slightly active at this dilution whereas some substance augmenting infectivity was not.

Increased infectivity from dilution of juices from plants infected by PVX.—Plants of *D. metel, Chenopodium amaranticolor,* and *Capsicum frutescens* var. Wisconsin Lakes were inoculated with PVX. The disease developed very slowly in Chenopodium and Capsicum, and lesions were few. Juice was obtained from symptomatic leaves of each of the 3 species 9, 14, and 17 days, respectively, after inoculation. Undiluted juice and 7 dilutions of each juice, as well as 7 dilutions of the stock inoculum, were assayed on *G. globosa.* Three such tests were made, with 8 replications in each.

Moderate dilution increased the infectivity of each of the test juices, an indication that inhibitors were present in each juice (Fig. 1-B). The infectivity of

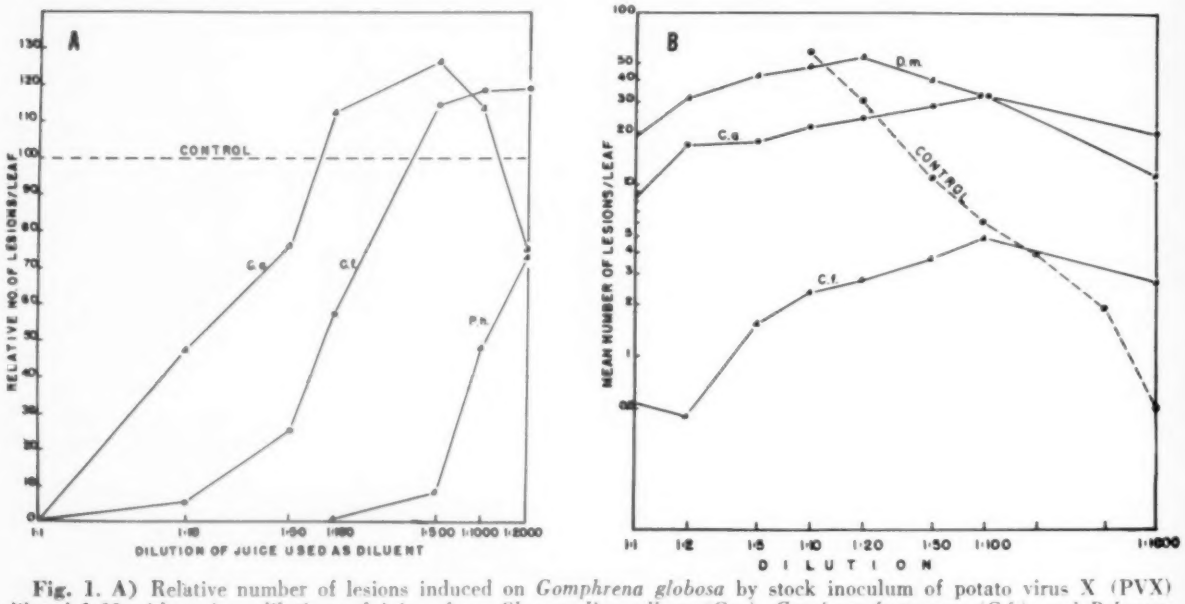

Fig. 1. A) Relative number of lesions induced on *Gomphrena globosa* by stock inoculum of potato virus X (PVX) diluted 1:10 with various dilutions of juices from *Chenopodium album* (C.a.), *Capsicum frutescens* (C.f.), and *Pelargonium hortorum* (P.h.). The mean number of lesions per leaf induced by a 1:10 aqueous dilution of the stock inoculum (64, 27.3, and 26.6, respectively) was assigned the value of 100 in each test; this is indicated by the broken line. Each point is based on 24 leaves. **B)** Relative infectivity of different dilutions of juices from plants of *C. frutescens* (C.f.), *Chenopodium amaranticolor* (C.a.), and *Datura metel* (D.m.) infected by PVX. The control consisted of the stock inoculum of PVX (juice of infected tobacco). Each dilution was rubbed on 24 leaves of *G. globosa.*

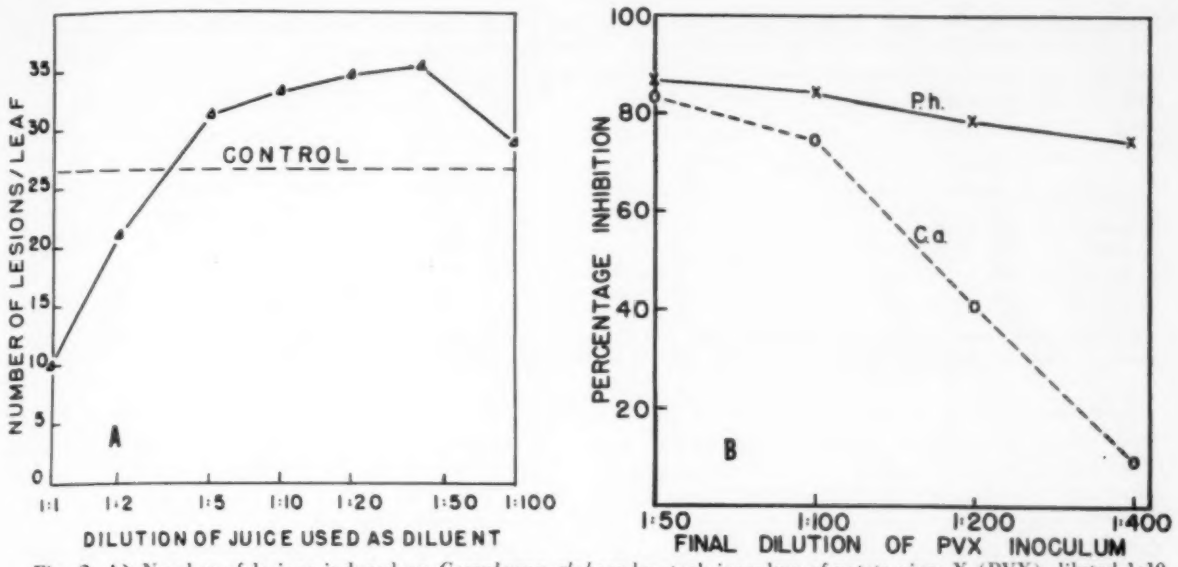

Fig. 2. A) Number of lesions induced on *Gomphrena globosa* by stock inoculum of potato virus X (PVX) diluted 1:10 with juice of *Nicotiana glutinosa* previously diluted with distilled water. **B)** Inhibition of PVX (as indicated by lesion formation on *G. globosa*) by juice of *Pelargonium hortorum* (P.h.) or of *Chenopodium amaranticolor* (C.a.) at a constant ratio of juice to virus at different dilutions. The final dilutions of inhibitory juice were the same as those of PVX stock inoculum in the C.a. series and 10 times as great in the P.h. series.

PVX, as measured by the number of local lesions, was highest in juice from *D. metel* at a dilution of 1:20 and highest in juice from Chenopodium and Capsicum at a dilution of 1:100. In each case, the slope of the dilution curve is considerably less than that for the control, an indication that these juices were still somewhat inhibitory at the higher dilutions used.

Occurrence of augmenters in plant juice.—In several experiments, some plant juices inhibited PVX infection when undiluted but enhanced virus infectivity when diluted (Table 1). Special tests confirmed this, although the magnitude of the augmenting activity appeared to depend largely on the age of the plants that provided the juice.

Fig. 2-A shows the results of one test with juice of medium-sized plants of *N. glutinosa*. Stock inoculum diluted 1:10 with 1:5 to 1:50 dilutions of the juice induced appreciably more lesions on *G. globosa* than did a comparable inoculum diluted with water. The consistency of such results, with this and with juices from other plants, indicates the presence of an augmenter or augmenters in the juices, rather than experimental error. Juices of *N. glutinosa* and *L. esculentum* also augmented lesion formation when 1:10 dilutions were used in watering the assay plants or were sprayed on plants before inoculation. In addition, juices from tomato and cucumber plants showed augmentive activity in the basic test (Table 1) but not when 1:10 dilutions of the juices were used as diluents (1:10) for juice of PVX-infected pepper plants, i.e. these juices had no augmenting effect in the presence of the inhibitory pepper juice.

Influence of heating on inhibition of plant juices.—The juice from leaves of relatively old potato plants of the variety Saco (immune from PVX and virus S) was heated 10 minutes in a water bath at various temperatures, cooled to about 25°C, and used as diluents for PVX. Twenty-four plants of *G. globosa* were inoculated. The inhibitory activity of Saco juice was decreased slightly by heat treatment at 50° and totally removed only by heat treatment at 80° (Table 2).

Results were similar in similar tests with juice of *L. esculentum* var. Bonny Best. In one series of tests, the inhibitory activity was totally removed by treatment at 60°; in another test, the inhibitory activity of juice from older plants was not completely removed at 80° Juices of some plants, in contrast, contained heat-stable inhibitors. After 10 minutes in boiling water, the juice of *P. hortorum* retained its full inhibitory activity, whereas juices of *Capsicum frutescens, Chenopodium amaranticolor*, and *Spinacia oleacera* lost a slight amount of inhibitory activity.

TABLE 2.—*Number of lesions induced by stock inoculum of potato virus X on Gomphrena globosa when juice from Solanum tuberosum var. Saco was used as diluent after being heated 10 minutes at various temperatures*

Diluent	Mean no. lesions/leaf[a]	Percentage of control
Water	26.6	100.0
Unheated juice	4.6	17.2
Juice heated at 32°C	4.6	17.2
Juice heated at 41°C	4.7	17.6
Juice heated at 50°C	8.2	30.8
Juice heated at 60°C	19.0	71.0
Juice heated at 71°C	25.0	93.9
Juice heated at 80°C	27.1	101.8

[a] Twenty-four leaves of *G. globosa* were rubbed with each treatment.

TABLE 3.—*Number of lesions produced on Gomphrena globosa and on Nicotiana rustica by potato virus X diluted with juice from different parts of Solanum tuberosum and of G. globosa*

Source of diluent	Assay plant	Mean no. lesions/leaf[a] with indicated diluent			
			Juice		
		Water (control)	From young leaves	From older leaves	From stems
S. tuberosum var. Cherokee	*G. globosa*	21.2	0.2	3.5	14.6
S. tuberosum var. Saco	*G. globosa*	9.1	0.2	1.1	11.0
G. globosa	*G. globosa*	30.2	6.9	13.9	
G. globosa	*N. rustica*	4.8	2.2	3.2	

[a] Figures are based on 24 leaves in both tests with juices from *S. tuberosum*, 36 leaves in tests with juices from *G. globosa* where *G. globosa* was the assay plant, and 48 half-leaves where *N. rustica* was the assay plant.

Inhibition of PVX by diluted juices at a constant ratio of test juice to virus.—Four dilutions of the juice of each of 2 plant species, *C. amaranticolor* and *P. hortorum*, were mixed with diluted stock inoculum so that the ratio of test juice to virus was the same for the 4 mixtures prepared with one kind of juice. They were then assayed on *G. globosa*.

At all dilutions used, juice of *P. hortorum* caused over 70% inhibition, but there was a consistent decrease in percentage of inhibition as the dilution was increased (Fig. 2-B). This trend was quite marked in tests with Chenopodium juice, with little or no inhibition at a dilution of 1:400.

The consistent decrease of inhibition with dilution suggests that neither inhibitor acts by combining with the virus; however, the sharp decrease in the one case, and the gradual decrease in the other, may indicate that different substances and perhaps different mechanisms are involved.

Inhibition by juice from different parts of the plant.—Observations indicated that the age of the source plants or leaves may greatly influence the inhibitory activity of juice. This possibility was tested in experiments with 2 potato varieties, Cherokee and Saco, and *G. globosa*. The juices from young top leaves, from older leaves, and from stems of potato were tested separately. With *G. globosa*, 2 lower pairs of leaves were treated as older leaves, and the 2 upper expanded leaf pairs plus the growing tip were treated as young leaves. Juice of *G. globosa* was tested on 2 assay plants, *G. globosa* and *Nicotiana rustica*.

In tests with potato juices, inhibition was greatest by juice from young leaves (Table 3), slightly less by juice from older leaves, and slight or absent by juice from stems. With *G. globosa*, the juice of young leaves was much more inhibitory on both assay plants than was juice from older leaves.

Inhibition of TMV by juices from 7 plant species.—Inhibition of TMV by juices from the species listed in Table 4 was also tested. The juices were obtained from plants approaching the flowering stage. The juices of *Capsicum frutescens* var. Wisconsin Lakes and *Chenopodium amaranticolor* were tested at a dilution of 1:10, and the juice of *P. hortorum* var. Richard at 1:100, since undiluted juices of these speci completely inhibited PVX.

The inhibitory activity toward TMV was high (Table 4). In general, the results were very similar to those obtained with PVX; however, the juice of *P. hortorum* appeared to be a little less inhibitory to TMV than it usually was to PVX. The differences may have been varietal, since the Pelargonium varieties in the 2 kinds of tests were different.

Inhibition by juices sprayed on assay plants before inoculation.—The juices of 5 plant species were tested for ability to inhibit when sprayed on the upper surfaces of leaves of *G. globosa* before inoculation with PVX. The source plants were relatively young and rapidly growing except for the pepper and spinach, which were in the early flowering stage. Juices were extracted from frozen plants of *C. album* and *P. hortorum* and from unfrozen plants of the other species; with *C. album*, the pulp was diluted 1:2 to facilitate extraction. Except for the Pelargonium juice,

TABLE 4.—*Number of lesions produced on Nicotiana glutinosa by tobacco mosaic virus diluted in juices from 7 plant species*

Source of juice	Dilution of juice	Mean no. lesions per half-leaf[a]	Percentage of control
Control (water)		187.5	100.0
Capsicum frutescens var. Wisconsin Lakes	1:10	17.2	9.1
Chenopodium amaranticolor	1:10	0.0	0.0
Datura metel	Undiluted	12.6	6.6
Pelargonium hortorum var. Richard	1:100	37.2	19.7
Solanum integrifolium	Undiluted	63.8	34.0
Solanum tuberosum var. Saco	Undiluted	25.3	13.4
Spinacia oleacera var. Bloomsdale	Undiluted	0.0	0.0

[a] Figures based on 24 half-leaves of *Nicotiana glutinosa*.

TABLE 5.—*Number of lesions induced by potato virus X on leaves of Gomphrena globosa sprayed with juices from 5 plant species 1, 3, or 7 days before inoculation*

Source of juice	Mean no. lesions[a] per leaf from inoculations made the indicated no. of days after spraying					
	1		3		7[b]	
	Not sprayed	Sprayed	Not sprayed	Sprayed	Not sprayed	Sprayed
Capsicum frutescens var. Wisconsin Lakes	17.2	0.0	29.2	0.0	9.0	0.0
Chenopodium album	*10.5*	0.2	*21.3*	1.3	*6.1*	0.6
Pelargonium hortorum	23.6	0.0	14.0	0.0	19.0	0.0
Phaseolus vulgaris var. Scotia	17.2	8.6	29.2	10.1	9.0	4.1
Spinacia oleacera var. Bloomsdale	17.2	3.4	29.2	8.9	9.0	2.7

[a] Figures in experiments with *C. frutescens*, *P. vulgaris*, and *S. oleacera* were based on 32 leaves each; those in tests with *P. hortorum* and *C. album* were based on 24 leaves except when italicized, in which case 8 leaves were used.
[b] In the test with juice from *C. album*, inoculation was made after 6 days.

which was diluted 1:2, the juices were sprayed undiluted on the leaves of *G. globosa*. The sprayed leaves were protected from wetting during subsequent watering of the plants. After 1, 3, or 7 days, the sprayed leaves were inoculated with a 1:10 aqueous dilution of the stock inoculum.

Inhibition by juices of *Capsicum frutescens*, *Chenopodium album*, and *P. hortorum* remained unchanged for at least 7 days (Table 5). Moreover, the juice of *P. hortorum* was still completely inhibitory at 20 days, the longest period tested. The juices of *P. vulgaris* and *S. oleacera* were only partially inhibitory, but they too were active for at least 7 days.

When the lower surfaces of leaves were sprayed and the upper surfaces inoculated 1 or 3 days later, some inhibition by juices of *P. hortorum* and *C. frutescens* was evident. In contrast, more lesions formed on leaves treated with juices of *P. vulgaris* or of *S. oleacera* than on untreated leaves; the respective numbers of lesions were 48% and 79% greater on treated leaves than on untreated leaves.

In another series, only one leaf of each opposite pair on each assay plant was sprayed with the juice of *C. album* or of *P. hortorum*. Respective inoculations were made on both leaves after 5 and 7 days. Untreated plants were inoculated as a check. In both cases, the number of lesions was about 20% smaller on untreated leaves of treated plants than on the check. Hence, there was little or no movement of the inhibitors to opposite leaves.

Inhibitory activity of stored plant juices.—When juices were harvested for the basic test, portions were stored at 4°C. After several weeks, some of these were tested by the usual procedure. Inhibitory activity declined slightly or not at all in juices from 6 plant species (*Capsicum frutescens*, *Chenopodium album*, *P. hortorum*, and others) but declined appreciably in juices from *D. stramonium*, *P. vulgaris*, and *P. floridana* (Table 6).

DISCUSSION.—Relatively few of the plant species used in this study have been tested as PVX inhibitors by other workers. The present results are in agreement with the report by Kuntz and Walker (12) of marked inhibition of PVX by spinach juice. Marked inhibition by juice of *C. frutescens* was reported by McKeen (15) and inhibition by Datura juice by Gendron and Kassanis (9).

As in most previous work (4, 12, 19), dilution proved effective in removing inhibitory activity. A dilution of 1:10 was sufficient in most cases, but the persistence of inhibition at much greater dilutions is not peculiar to the examples reported here. Kuntz and

TABLE 6.—*Lesions induced on Gomphrena globosa by potato virus X when juices from several plant species were used as diluents immediately after preparation and after storage at 4°C*

Source of juice	Storage time (days)	Lesion induction,[a] as percentage of control	
		Diluted with fresh juice[b]	Diluted with stored juice[c]
Capsicum frutescens var. California Wonder	79	0.0	0.5
Chenopodium album	77	1.9	1.8
Cucumis sativus	76	28.9	27.4
Datura stramonium	48	0.9	13.2
Nicotiana tabacum (Holmes' NN)[d]	59	32.0	33.5
Pelargonium hortorum	84	0.0	0.3
Phaseolus vulgaris var. Bountiful	81	9.1	18.3
Physalis floridana	89	28.7	69.1
Solanum integrifolium	31	8.5	10.2
Solanum tuberosum (USDA Seedling 41956)	48	32.0	23.1

[a] Each figure represents the mean number per leaf, based on 24 leaves.
[b] Data from basic tests (Table 1).
[c] Mean number of lesions per leaf for the control was 27.7 in the test with 7 juices and 14.6 in the test with the other 3.
[d] Containing the *N* gene from *N. glutinosa* (11).

Walker (12) found spinach juice inhibitory to TMV at dilutions of 10^{-3} to 10^{-4}. Crowley (6), working with cucumber seed extracts, reported that virus activity was not wholly restored by dilution; however, he used a buffer in the control inoculum, which may have enhanced virus infectivity. Although moderate dilution did not always completely remove the inhibitory activity of juices, the apparent infectivity of PVX diluted with a 1:10 dilution of juice was never less than that of PVX diluted with undiluted juice. Consequently, detection of PVX (and perhaps other viruses) appears more probable in moderately diluted juices than in undiluted juices. This would be particularly true with such source plants as pepper, whose juice is very inhibitory and in which the apparent virus concentration is quite low (Fig. 1-B).

The present studies, as well as other reports, indicate that heat treatments remove inhibitory activity in some cases and not in others. Our results indicate that most of the PVX inhibitors that resist dilution also resist heat, but this was not true in all cases and apparently is not necessarily true for plant virus inhibitors in general. Thus, the TMV inhibitor in spinach juice resists dilution but is destroyed by 12 minutes at 70°C (12). An inhibitor of cabbage mosaic virus (cabbage virus A) in spinach juice is not destroyed by 15 minutes at 125°. This inhibitor also resists dilution (12).

It is evident from the present work and from previous reports (5, 12, 15, 20) that heat treatment of inocula can be a valuable tool in virus research. A good example is the recent work of Bagnall, Larson, and Walker (1), who found that the infectivity of the interveinal mosaic virus (a strain of leafrolling mosaic virus) in potato juice was markedly increased by 10 minutes at 50° and that juice from PVX-infected tobacco var. Samsun heated at 40–50°C produced more local lesions on *G. globosa* than unheated control juice.

Most of the juices tested in the present study (including juice of *Cucumis sativus*) retained inhibitory activity to PVX after prolonged storage at 4°. Weintraub and Willison (20), however, reported that juices of 4 species of curcurbits lost their ability to inhibit certain stone fruit viruses within 4–8 hours. Spinach juice, in contrast, lost none of its ability to inhibit any of several viruses during 15 months at room temperature (12). Some inhibitors are very stable even when exposed to air, for certain juices were highly inhibitory even if sprayed on leaves several days before inoculation.

Since all of the test juices inhibited PVX to some extent, inhibitors appear to be widely distributed in herbaceous species; there is no indication, however, that all these juices contain the same type of inhibitor or that the mechanism is the same in all cases. With slight inhibition that disappeared with moderate dilution, it may be that no specific material or specific mechanism is involved. Quite possibly, any of several normal constituents of such plant juices may be phytotoxic, particularly when introduced into a wound and not removed by rinsing. With juices of Pelargonium, Capsicum, and Chenopodium, however, other types of materials and/or mechanisms appear to be involved. Thus, the inhibitors in juices of these plants not only are resistant to dilution and to heat but also are effective when applied to leaves many days before inoculation. The data are consistent with the idea expressed by some workers (2, 3, 6) that inhibitors act on the host plant rather than on the virus. It is apparent, however, that the inhibitor in Pelargonium juice differs from that in the other juices tested. Juice of this plant is much more resistant to dilution, and its potency did not diminish greatly with dilution when the inhibitor-virus ratio was held constant. Since the Pelargonium inhibitor is very potent and quite stable, and protects sprayed leaves for long periods, this material should receive additional study.

Our results with juices applied to the upper surfaces of leaves before inoculation agree with those of McKeen (15), who found that juice of *C. frutescens* applied to assay plants up to 24 hours before inoculation prevented infection. When juice was applied to the lower surfaces of leaves before inoculation, McKeen observed a high degree of inhibition whereas we observed little or none.

The presence of augmenters in certain plant juices has been indicated in previous reports. Thus, Benda (5) obtained evidence of an augmenter in the juice of New Zealand spinach that had been boiled to remove inhibitors. The milder heat treatments of the present work did not reveal augmenters in the juices tested, but such evidence was obtained in tests with juices diluted sufficiently to remove their inhibitory activity. Benda (5) also reported that sodium oxalate acts as an augmenter and that it may act by virtue of its antagonistic action on calcium ions on the surface of the protoplast. This mechanism, or similar ones, may be involved in the results reported herein; however, other factors, such as surface tension effects, pH, and certain salts, may be involved.

Department of Plant Pathology
Cornell University
Ithaca, New York
and
Department of Plant Pathology
University of Wisconsin
Madison, Wisconsin

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PHYSIOLOGIC SPECIALIZATION IN PERONOSPORA MANSHURICA [1]

Don F. Grabe and John Dunleavy [2]

SUMMARY

Two new races of *Peronospora manshurica*, the causal agent of downy mildew of soybeans, are described. Race 7 was isolated from soybean seeds grown in Illinois. Race 8 was isolated from soybeans grown in Indiana, Missouri, and 3 locations in Iowa. Proposed for differentiation of races of *P. manshurica* are a set of 14 soybean varieties: Illini, Richland, Mukden, Wabash, Pridesoy, Norchief, Laredo, S100, CNS, F.C. 33123,[3] Palmetto, Acadian, Ogden, and Roanoke. Five infection types were established for rating varietal reaction to infection by the fungus. In addition to the isolation of races 7 and 8, race 1 was obtained from seed grown in Arkansas, and race 2 from seed grown in Iowa. Since these 2 races were originally differentiated on only 3 varieties, these descriptions have been amended to include their reactions on the entire set of 14 differentials.

Physiologic specialization in *Peronospora manshurica* (Naum.) Syd. ex. Gäum. was first demonstrated in 1950, by Geeseman (1). He differentiated 3 collections of the fungus as races 1, 2, and 3 by their reaction on Richland, Illini, and any 1 of 5 other midwestern varieties. Lehman (3) reported race 4 from North Carolina, and later (4) designated other isolates from North Carolina as races 3A, 5, 5A, and 6. The number of differential varieties was increased to 10 for distinguishing these latter races. In 1955, a study (2) was begun to investigate the possibility of using various races of the downy mildew fungus for identifying soybean varieties. During the course of this study, additional races were discovered.

METHODS.—Soybean seeds encrusted with oospores of *P. manshurica* were obtained from seed samples on file in the Iowa State College Seed Laboratory and from the Georgia, Indiana, and Missouri Agricultural Experiment Stations. In addition, conidia were obtained from infected plants in the field near Ames, Iowa.

Systemically infected plants were obtained from these oospore-encrusted seeds by 4 methods: 1. Encrusted seeds were planted in pots in a cool greenhouse and allowed to germinate and grow slowly after the methods suggested by Lehman (4). 2. A water suspension of oospores scraped from the seed coats was placed between the cotyledons of sprouting seeds before they were planted in soil. 3. Oospores were mixed with talc and placed between the cotyledons of sprouting seeds. 4. Moistened oospore-encrusted seeds were held 6 days at 10°C before being planted in the greenhouse. All 4 methods were uniformly

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[2] Formerly Associate Iowa State College, now Assistant Agronomist, Department of Agronomy, Mississippi State University; and Associate Professor, Department of Botany and Plant Pathology, Iowa State College, and Plant Pathologist, Crops Research Division, ARS, U. S. D. A.

[3] U. S. Department of Agriculture Field Crops seed acquisition number.

successful, resulting in an average of nearly 20% systemically infected seedlings.

No conidia were produced on infected leaves in the greenhouse, thus there was no danger that the races would be mixed. To obtain conidia for infection experiments, leaves with lesions about 3 weeks old were removed from systemically infected plants and placed on wet filter paper in a Petri dish and held at 18°C. Sporulation occurred overnight.

The supply of inoculum was increased on seedlings of susceptible varieties growing in 4-in. pots. Conidia were washed from systemically infected leaves into water containing 0.1% Tween-20 and sprayed over the seedling leaves with an air-pressure sprayer. The inoculations were made when the primary leaves were about 3/4 unfolded (8–10 days after planting when grown at 75–80°F). The inoculated seedlings were held 24 hours at 65°F. The plants were kept moist during the infection period with quart-size plastic bags placed over each pot. Infected areas sporulated in about 10 days on plants held at 70–75°F during the incubation period, and in 7–9 days on plants grown at 80–85°F.

Physiologic specialization of the various isolates was determined on a set of 14 differential soybean varieties. Three of the varieties—Illini, Richland, and Mukden—were used by Geeseman (1) in differentiating races 1, 2, and 3. Seven additional varieties—Roanoke, Acadian, CNS, Ogden, Palmetto, F.C. 33123,[3] and S100—were added to the original 3 varieties to help distinguish southern races of the fungus. Four other varieties—Laredo, Wabash, Pridesoy, and Norchief—were added to the above 10 to help distinguish the new isolates.

The differential varieties were grown in the same flat for each test. Inoculation procedures were similar to those described previously. Moisture was maintained on inoculated plants by covering the seedlings with an overturned flat, which in turn was covered with wet burlap bags. The flats were then covered with a plastic sheet to prevent loss of moisture.

In 1955–56, seedlings were inoculated with conidia

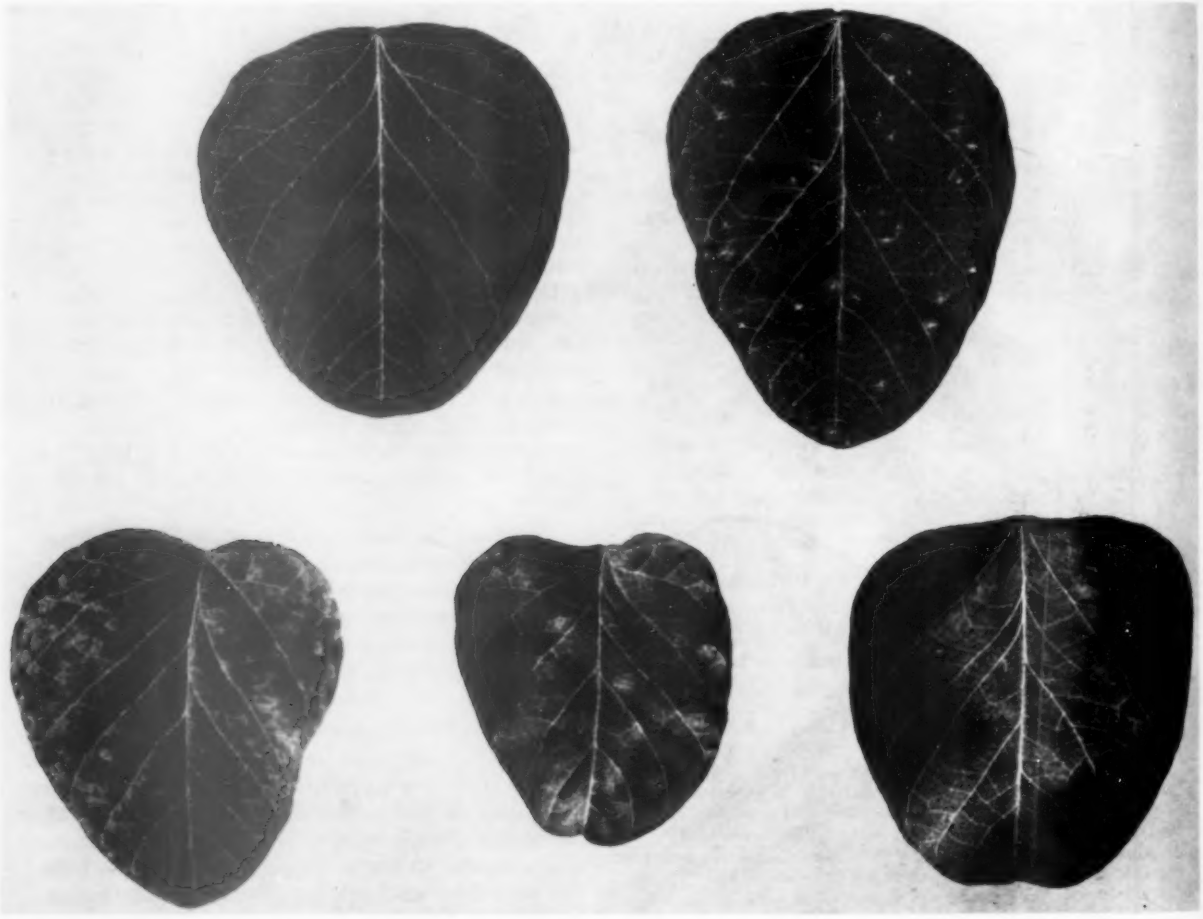

Fig. 1. Infection types of downy mildew of soybeans. **Top row,** left to right: type 1, immune; type 2, small flecks. **Bottom row,** left to right: type 3, small discrete chlorotic areas, irregular in shape, up to 2 mm in diameter; type 4, chlorotic areas up to 4 mm in diameter, sometimes merging, often delimited by veins, presenting an angular appearance; and type 5, large confluent chlorotic areas uniformly covering much of the leaf. Types 1 and 2 are considered resistant; types 3, 4, and 5 are susceptible.

TABLE 1.— *Disease reactions of 14 differential varieties of soybeans to 4 races of P. manshurica*

Varieties	Races			
	1	2	7	8
Illini	5	5	4	5
Richland	5	5	1	5
Mukden	1	4	4	4
Wabash	1	4	4	4
Pridesoy	5	1	1	4
Norchief	1	4	1	5
Laredo	5	5	1	5
S100	4	4	4	5
CNS	4	4	4	4
F.C. 33123	1	2	3	3
Palmetto	1	1	1	1
Acadian	1	1	1	1
Ogden	1	1	1	1
Roanoke	1	2	1	2

originating from several systemically infected leaves of a collection. In 1956–57, single-spore isolates of each collection were increased and maintained for the screening tests.

The reaction of each variety to the fungus was recorded 10 days after inoculation. A severity rating scale based on infection types (Fig. 1) was established as follows: 1, immune; 2, small flecks, less than 0.5 mm in diameter; 3, small discrete chlorotic areas, irregular in shape, up to 2 mm in diameter; 4, chlorotic areas up to 4 mm in diameter, sometimes merging, often delimited by veins, presenting an angular appearance; 5, large confluent chlorotic areas uniformly covering much of the leaf.

RESULTS.—The reactions of the 14 differential varieties to 4 races of downy mildew are indicated in Table 1. The isolate from Arkansas reacted the same as Geeseman's race 1 on Illini, Richland, and Mukden. However, since Geeseman differentiated his races on only these 3 varieties, it is impossible to determine what their reactions might have been on the extended list of 14 differentials. Therefore the description of race 1 has been amended to include the reactions of the entire set of 14 differential varieties. Similarly, the description of race 2 of Geeseman has been amended to include the reactions of the isolate from Cresco, Iowa, on the entire set of differentials.

The isolate from Illinois should be considered a new race of *P. manshurica*, race 7, which differs from race 2 in its failure to infect Richland, Norchief, and Laredo.

The isolates from Indiana, Missouri, and 3 locations in Iowa make up still another group, race 8. This race appears similar to race 2, but race 8 infects Pridesoy while race 2 does not. Kabott, Goldsoy, and Pagoda were also resistant to race 2 and susceptible to race 8.

The reactions of 80 varieties to races 7 and 8 have been published in a separate paper (2).

DISCUSSION.—The work of Lehman (4) and the present work clearly indicate that there is considerable variation in the ability of isolates of *P. manshurica* to attack specific varieties of soybeans. Increased soybean production in the United States has brought soybean fields much nearer to one another, with a consequent more rapid spread of downy mildew. With increased production there is also the possibility of 2 or more races occurring in the same area. There is a great need for more exact information on race identity in the various soybean-producing regions of the country so that breeding for resistance to downy mildew can proceed on a more sound basis.

Using single-spore isolates for race identification is mandatory with a fungus as variable as *P. manshurica*. Although all single-spore isolates in this study gave the same disease reaction as the isolates from which they were taken, several isolates have been found that are obvious race mixtures. Special care should be taken when isolates are obtained from soybean-breeding or disease nurseries because seed from numerous sources is usually sown in such nurseries and the probability of race mixtures is high.

Minor differences in pathogenicity were noted between isolates of some races; but, since no practical purpose would be served by identifying such variants, no attempt was made to differentiate subraces.

A factor hindering studies of the race situation is an inability to preserve cultures of the fungus once they are isolated. At present, the only method of accomplishing this is to inoculate new seedlings every 8–10 days or to obtain systemic infection from conidia. Attempts to obtain systemic infection with conidia never yielded more than 1% infection. A better method of preserving conidia is needed. Techniques of freezing that are successful on other downy mildews have so far been unsuccessful on *P. manshurica*. A supply of viable conidia is desirable for distribution of inoculum of specific races to other investigators and for screening varieties and selections for resistance to the pathogen.

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DIFFERENTIAL HOST RANGE OF THE MONOCARYON AND THE DICARYONS OF A EU-AUTOECIOUS RUST[1]

H. H. Flor

SUMMARY

Twenty-five differential varieties of flax, monogenic for resistance, were subjected to infection by sporidia derived from a urediospore culture of race 210 of the flax rust fungus, *Melampsora lini*. Normal pycnia and aecia developed on the 6 varieties to which the urediospore culture was either homozygous or heterozygous for high virulence. Only flecks or minute pycnia developed on varieties to which the urediospore culture was moderately virulent or moderately avirulent. With the exception of Bombay, all varieties to which the urediospore culture was highly avirulent were apparently immune. Bombay, immune from the urediospore culture, was highly susceptible to the sporidia, but was immune from aeciospores that developed on Bombay and from the F_2 urediospore cultures derived from aecia that developed on it. In being susceptible to the monocaryon and immune to the dicaryon, Bombay served as an alternate host to race 210 of *M. lini*, a eu-autoecious rust.

Arthur (1) showed that *Melampsora lini* (Ehrenb.) Lév. was eu-autoecious. Flor (2) found it to be highly specialized on varieties of *Linum usitatissimum* L. He later (3) developed 25 lines of flax, monogenic for resistance, that served to differentiate physiologic races of the flax rust fungus.

In the North Central States, flax rust overwinters in the telial stage. On flax varieties susceptible to the prevalent races, pycnia and aecia are usually observed a week to 10 days before uredia. In 1957, a heavy infection of pycnia and aecia developed on the variety Bombay in experimental plots at Fargo, N. D., but no uredia or telia. Bombay was immune to all collections of flax rust made in 1957, including those obtained from the experimental plots. This immunity indicated that the host range of the sporidia (monocaryon) differed from that of the aeciospores and the urediospores (dicaryons).

MATERIAL AND METHODS.—Telia of a culture of race 210 of the flax rust fungus, a race prevalent in the North Central States, were developed in the greenhouse. When the teliospores had been induced to germinate, the 25 differential varieties of flax were sown in 4½-in. pots. Four seeds of each of 3 differentials and of Bison were sown at 90° intervals near the edge, and 4 seeds of Bombay at the center of each pot. The seedlings were inoculated at about the 4-in. stage by suspending germinating teliospores over them in bell jars. Only two bell jars were used; so the pots of seedlings were successively subjected to the sporidial shower for 6–12 hours. After removal from the bell jars the inoculated seedlings were placed in a moist chamber for 12 hours. Sporidial production diminished after 36–48 hours, and the telium-bearing straw was washed and then dried for 24 hours before the second replicate of 25 differential varieties was inoculated. The seedlings were then about 6 in. tall.

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RESULTS.—The pathogenicity of the urediospore culture of race 210 and that of the sporidia and F_2 urediospore cultures obtained from it on 25 differential varieties are shown in Table 1. In each of the two replicates, normal pycnia developed on Bison, Koto, Burke, and Ward, varieties susceptible to race 210, and on Bombay, Wilden, and Barnes, varieties resistant to it. Only flecks developed on Williston Brown, a variety moderately susceptible to the urediospore culture. Minute pycnia developed on the moderately resistant variety Cortland. A total of 464 pycnia de-

TABLE 1.—*Pathogenicity of urediospores, sporidia, and F_2 urediospore cultures of race 210 of M. lini*

Differential variety	Pathogenicity[a] on indicated differential variety of		
	Urediospores	Sporidia	F_2 cultures
Ottawa 770B	A	A	A
Dakota	A	A	A
Bombay	A	V	A
Stewart	A	A	A
Cass	A	A	A
Koto	V	V	V
Clay	A	A	A
Polk	A	A	A
Birio	A	A	A
Kenya	A	A	A
Akmolinsk	A	A	A
Abyssinian	A	A	A
Leona	A	A	A
Wilden	A	V	H[b]
Williston Brown	MV	A	MV
Victory A	A	A	A
Bison	V	V	V
Bolley Golden sel.	A	A	A
Barnes	A	V	H[b]
Towner	A	A	A
Marshall	A	A	A
Cortland	MA	A	MA
Pale Blue Crimped	A	A	A
Burke	V	V	V
Ward	V	V	V

[a] A = avirulent; MA = moderately avirulent; V = virulent; MV = moderately virulent; and H = heterozygous.

[b] On Wilden and Barnes 32 F_2 cultures were avirulent and 11 were virulent.

veloped on the 64 plants of Bison, and 553 pycnia developed on the 71 plants of Bombay subjected to sporidial infection.

Bombay was immune from aeciospores produced on it, from the 38 F_2 urediospore cultures obtained by selfing pycnia that developed on it, and from the 5 F_2 urediospore cultures obtained by selfing pycnia on Bison. Wilden and Barnes were susceptible to 10 of the 38 F_2 cultures developed on Bombay and to 1 of the 5 F_2 cultures developed on Bison. The selfed F_2 cultures had the pathogenicity of the parental urediospore culture on all the other differential varieties. The segregation of the F_2 cultures for pathogenicity on Wilden and Barnes approximated the ratio of 3 avirulent to 1 virulent expected if the parent culture of race 210 was heterozygous for the dominant genes conditioning avirulence on these varieties. In mutation studies (4) all mutants to virulence on Wilden attacked Barnes also, indicating that the genes conditioning pathogenicity on these varieties are closely linked.

S'Jacob (5) reported that some races obtained in the Netherlands did not attack the leaves of Bombay but produced a susceptible reaction (teliospores) only on the stem. This susceptibility only to stem infection was unique to Bombay, as was its susceptibility to sporidia of a race to whose aeciospores and urediospores it was immune. These peculiarities of Bombay may indicate that it differs from the other differential varieties of flax in mechanism or nature of resistance.

In being susceptible to the monocaryotic stage and immune to the dicaryotic stages, Bombay plays a role in the life cycle of the culture of race 210 of *M. lini* somewhat comparable to the role of an alternate host in the life cycle of heteroecious rusts. However, the pycnial and aecial stages of the culture developed normally on varieties susceptible to it; so Bombay is not essential to completion of the life cycle of the rust. The culture might be termed "facultatively heteroecious," and this may be an intermediate step in the evolution of heteroecism.

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COLLAR ROT OF PEAR TREES IN BRITISH COLUMBIA [1]

D. L. McIntosh [2]

SUMMARY

Phytophthora cactorum causes death of the bark of pear trees close to ground level. Losses have been most numerous among 5- to 6-year-old d'Anjou trees although Bartlett trees of the same age are also quite susceptible. There may be little or no visible effect on the vigor or fruit size of a diseased tree during the season in which infection occurs, even though its trunk is girdled almost completely by the infection. Trees younger than fruiting age (3–4 years) proved to be more resistant than trees of fruiting age (5–6 years) when artificially inoculated.

A new disease affecting young d'Anjou and Bartlett pear trees in an orchard near Kelowna, British Columbia, was reported to the Plant Pathology Laboratory, Summerland, in March, 1957. The bark of several 5-year-old trees had rotted from just below ground level to a height of 10–12 in. (Fig. 1-A). There was no obvious damage to the crown or roots of some of the trees, although the tops were dead, apparently as a result of the injury to the trunk. The orchard manager stated that the trees had grown satisfactorily the previous summer, having produced 12–18 in. of terminal growth, and fruit of good size.

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[2] Plant Pathologist, Plant Pathology Laboratory, Summerland, British Columbia, Canada.

Symptoms of the disease.—An examination of young trees for signs of the disorder in the early stages of its development was begun in June. When trucks of apparently healthy d'Anjou and Bartlett trees were examined close to ground level, two types of injury were found. In many of the trees necrotic tissue was found in the bark in patches of about 1–3 in. diameter or in narrow bands about the trunk. This tissue was dark brown to black, firm, usually shallow; it was sharply delimited from the apparently healthy bark, and often separated readily from the underlying tissue. Usually there was no indication on the surface of the bark that the tissue underneath was diseased. In more severely affected trees, the diseased bark extended inward to the cambium, girdling the trunk in a band several inches wide. It was not apparent during this survey that these two types of injury were symp-

Fig. 1. Symptoms of collar rot of pear trees. **A)** 6-year-old d'Anjou naturally infected. **B)** 7-year-old d'Anjou 6 weeks after it was inoculated artificially at ground level.

toms of the same disorder, nor could this be established in later tests. However, both types were more common in d'Anjou than in Bartlett.

The lesions in a few trees appeared to be enlarging. The margin of diseased tissue was not sharply defined from healthy tissue and varied in color from reddish brown to tan. These infections were similar in appearance to those caused by *Phytophthora cactorum* (Leb. & Cohn) Schroet., in other kinds of fruit trees.[3,4]

There was little or no indication, either in foliage color or in the length of terminal growth, that this damage to the trunk had affected the vitality of the tree, except in those trees that appeared to have been girdled prior to the 1957 season. Foliage was a lighter green on these trees than on healthy trees. Some trees with large areas of diseased bark had developed 12 in. or more of new terminal growth by the end of July.

THE PATHOGEN.—*Isolation.*—Many attempts were made to isolate a pathogen on nutrient agar during the dormant season, soon after the disease was reported, but none were successful. Later, several isolations from each of 18 diseased d'Anjou and 4 diseased Bartlett trees were made in the orchard during June, July, and October, by placing blocks of tissue from the margin of each type of lesion in apple fruits. One of the fruits, inoculated in July with tissue from a lesion that appeared active, developed a rot from which *P. cactorum* was recovered in pure culture. The characteristics of this isolate in culture were indistinguishable from those of *P. cactorum* isolated from other tree fruit sources.

Proof of pathogenicity.—D'Anjou and Bartlett trees were inoculated with cultures of the fungus grown for 14 days on a medium of corn meal and vermiculite, by removing a strip of bark 3/16 in. wide and about 1 in. long, placing inoculum next to the cambium, and replacing the strip. At first, when 2-year-old d'Anjou trees were inoculated, there was some necrosis of surface tissues around the inoculation wound, but the superficial lesion that developed did not resemble in any way the original lesion from which the fungus was isolated. This result was somewhat unexpected, but it was consistent with those of previous resistance trials[3] in which young Bartlett and d'Anjou trees proved resistant to *P. cactorum*. However, when the inoculations were repeated with 6-year-old trees, i.e., trees the same age as those infected naturally, the trunks were almost completely girdled in 6 weeks. Fig. 1-B shows the symptoms produced by July 21 in a d'Anjou tree inoculated June 9.

This apparent difference in susceptibility between recently planted trees and those of bearing age was observed in another trial, in which trees 3 and 4 years old not yet bearing fruit, and trees 6 and 7 years old that bore a small crop, were inoculated. Seven weeks later the infection in the nonbearing trees extended $\frac{1}{4}$–$\frac{3}{4}$ in. on each side of the wound and appeared to have stopped enlarging, while the trunks of some of the older trees were three-quarters girdled and the infection had spread as much as 6 in. upward from the point of inoculation. The size of the lesions in many Bartlett trees could not readily be determined unless the surface of the bark was removed to reveal the extent of infected tissue beneath. Occasionally, in favorable light, a slight difference in color between healthy and diseased areas of bark was seen on the surface. Diseased d'Anjou bark was recognized more readily—by slight swelling beyond the limit of the lesion, by the formation of longitudinal fissures, and by peeling of the epidermis. *P. cactorum* was reisolated from these trees.

This is the first time that natural infection of pear trees by *P. cactorum* has been recorded, although the crown rot disease has affected other kinds of fruit trees in British Columbia for many years. The possibility that this new isolate was more pathogenic to pear trees than those recovered previously from other tree fruit sources was investigated by inoculating fruiting Bartlett and d'Anjou with cultures of *P. cactorum*, isolated at intervals over 20 years from apple and cherry trees and from apple and peach fruits and maintained on potato-dextrose agar in the laboratory. Each isolate caused infection, and although there were differences in the size of lesions produced, there was no substantial evidence that the new isolate was more pathogenic to pear trees than were the others.

DISCUSSION.—It was concluded from previous trials

[3] McIntosh, D. L. 1953. A trunk and crown rot of sweet cherry in British Columbia. Phytopathology 43: 402-403.
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in which 2-year-old pear trees were inoculated with *P. cactorum*,[5] that the Flemish Beauty and Gorham varieties were quite susceptible and that Bartlett and d'Anjou were resistant. The results of this study show that the Bartlett and d'Anjou varieties are resistant only in the years before they begin to bear fruit, and that they are quite susceptible after the first crops are borne. Obviously, the reactions of young nonbearing trees to inoculation with *P. cactorum* are not reliable criteria of varietal resistance, and final assessments of resistance should be made on trees of fruiting age.

Bartlett and d'Anjou are the principal pear varieties grown in British Columbia. The losses from collar rot in recent years show the need for a resistant rootstock on which these varieties can be topworked.

Tree losses can be avoided if the disease is detected in the early stages of its development and the surface tissues are scraped from infected bark. Advance of the fungus is retarded or stopped in tissue exposed to the air and allowed to dry. The fungus may be active in a lesion for one season only, so that diseased trees may suffer no permanent damage if killing of the cambium is prevented.

PLANT PATHOLOGY LABORATORY
SCIENCE SERVICE, CANADA DEPARTMENT OF AGRICULTURE
SUMMERLAND, BRITISH COLUMBIA

HYBRIDIZATION BETWEEN RADIATION-INDUCED MUTANTS OF TWO VARIETIES OF DIAPORTHE PHASEOLORUM [1]

J. T. Threinen, Thor Kommedahl, and R. J. Klug [2]

SUMMARY

Separation of 2 varieties of *Diaporthe phaseolorum* (var. *caulivora* and var. *sojae*) on the basis of morphological differences in culture and differences in pathogenicity on soybeans is considered invalid. Nonsporulating mutants of both varieties resulted from irradiation. Growth of mutants varied on different media, but self-sterility resulting from irradiation did not appear to be the result of a nutrient deficiency. Self-sterile mutants of the variety *caulivora* behaved in crosses with parent types of both varieties as if heterothallic, indicating that the homothallism ascribed to the variety *caulivora* is not a constant character. Upon crossing mutants of the variety *caulivora* with the variety *sojae*, the perithecia produced had the morphological characters of the variety *sojae*. Most mutants were less pathogenic than the parents on soybeans; some were equally pathogenic. Mutants of the stem-canker variety *caulivora* produced symptoms on soybeans that were indistinguishable from symptoms produced by the pod-and-stem-blight variety *sojae*, making this an unreliable character for varietal separation.

INTRODUCTION.—Harter (6), in 1917, determined that *Diaporthe phaseolorum* (Cke. & Ell.) Sacc. was the cause of pod blight of Lima bean. *D. sojae* Lehman was described later (9), but this was renamed *D. phaseolorum* (Cke. & Ell.) Sacc. var. *sojae* (Lehman) Wehm. (12). In 1947, Luttrell (10) found *D. phaseolorum* to be limited to Lima beans whereas the variety *sojae* had a more extensive host range.

Welch and Gilman (13) reported that the fungus causing pod and stem blight of soybean was heterothallic and produced scattered single perithecia and typical phomopsis conidia (stylospores), and this fungus was called *D. phaseolorum* (Cke. & Ell.) Sacc. var. *sojae* (Lehm. & Wehm.). On the other hand, the fungus causing stem canker of soybean was homothallic, produced perithecia in caespitose groups, and lacked conidia, and this was called variety *batatatis* (Harter & Field) Wehm. The latter variety was reported by Athow and Caldwell (1) to be nonpathogenic to soybeans and different morphologically from the stem canker fungus, which they named *D. phaseolorum* (Cke. & Ell.) Sacc. var. *caulivora* Athow & Caldwell. Production of conidia and pycnidia by this variety has since been reported (4, 8).

The chief characteristics upon which the varieties *sojae* and *caulivora* are now differentiated are morphology and pathogenicity on soybean (*Glycine max* (L.) Merr.). To ascertain whether these differences are consistent throughout the range of variation of both varieties, studies were made with the parent types and irradiation-induced mutants from each of the two varieties of *D. phaseolorum*.

MATERIALS AND METHODS.—Cultures of *D. phaseolorum* var. *caulivora* were obtained from K. Athow and J. Tuite, Lafayette, Indiana, and A. A. Hildebrand, Harrow, Ontario, Canada. The organism was also isolated from soybeans at St. Paul, Minnesota.

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[2] Former Research Assistant, Associate Professor, and former Research Fellow, respectively, University of Minnesota.

[5] McIntosh, D. L., and Frances C. Mellor. 1953. Crown rot of fruit trees in British Columbia II. Rootstock and scion resistance trials of apple, pear, and stone fruits. Can. J. Agr. Sci. 33: 615-619.

Cultures of *D. phaseolorum* var. *sojae* were isolated from soybean stems grown in Florida, Mississippi, Iowa, and Minnesota.

Cultures were single-spored for 3 successive generations by the method described by Welch and Gilman (13). The Minnesota isolates were used for irradiation studies. Ascospores, conidia, and hyphal tips were irradiated with ultraviolet light or with polonium 210 (Po^{210}). Mutants morphologically different from the parent types were selected.

Exposure time ranged from 1 to 60 minutes for ultraviolet light, and 30 to 120 minutes for Po^{210}. Irradiation treatments were replicated 5 times for each exposure period.

Mutants S-13, S-14, and S-27 of *D. phaseolorum* var. *sojae* were obtained by irradiating conidia with Po^{210}. All other mutants of variety *sojae* were obtained by irradiating conidia with ultraviolet light.

Mutants of *D. phaseolorum* var. *caulivora* were obtained by irradiation with Po^{210}. Mutant C-2 was obtained by irradiating hyphae with Po^{210}. Mutants C-3, C-4, C-8, C-9, and C-10 were obtained by irradiating conidia. Mutants C-5, C-6, and C-7 were obtained by irradiating ascospores. Several other mutants were secured by subculturing sectors from initial mutants.

The mutants of *D. phaseolorum* var. *sojae* and var. *caulivora* were selected on the basis of increased or decreased fertility. This loss or gain of the ability to produce fruiting bodies and sporulate was frequently accompanied by changes in growth rate and mycelial color. These morphological changes were secondary in the selection process, however. Other mutants might have been chosen had another person made the selection.

RESULTS.—*Compatibility of the parent types and mutants of the varieties caulivora and sojae.*—The work on *Chaetomium* indicates that heterothallism in ascomycetes previously described as homothallic may not be uncommon (3). It seems possible that homothallism in certain fungi can be altered to heterothallism by irradiation. Attempts were made to cross self-sterile mutants of *D. phaseolorum* var. *caulivora* with mutants of *D. phaseolorum* var. *sojae*, with the parent types of both varieties, and *inter se* to determine whether mutants of the variety *caulivora* would behave as heterothallic organisms. Attempts were also made to cross mutants of the variety *sojae* with the parent type to determine whether mating types had been changed by irradiation.

Crosses were attempted in two ways: 1) Autoclaved soybean stems were placed on water agar in Petri plates and inoculated—at one end with cultures of the mutants and at the other end with cultures of the parents. Perithecia formed in 18 days on the stems. 2) Four-day-old mycelial cultures of mutants from the variety *caulivora* were spermatized with conidial suspensions of the parent types of the variety *sojae* and the variety *caulivora*. Conidial suspensions were prepared by dispersing ooze from pycnidia into autoclaved water. These suspensions were sprayed over 4–5-day-old cultures of mutants from the variety *caulivora*. Pycnidia of the parent type of this variety were secured by growing this organism on sweet clover stems. The cultures were incubated 18 days and observed for perithecia. Controls were sprayed with sterile water.

The spermatization procedure was repeated using

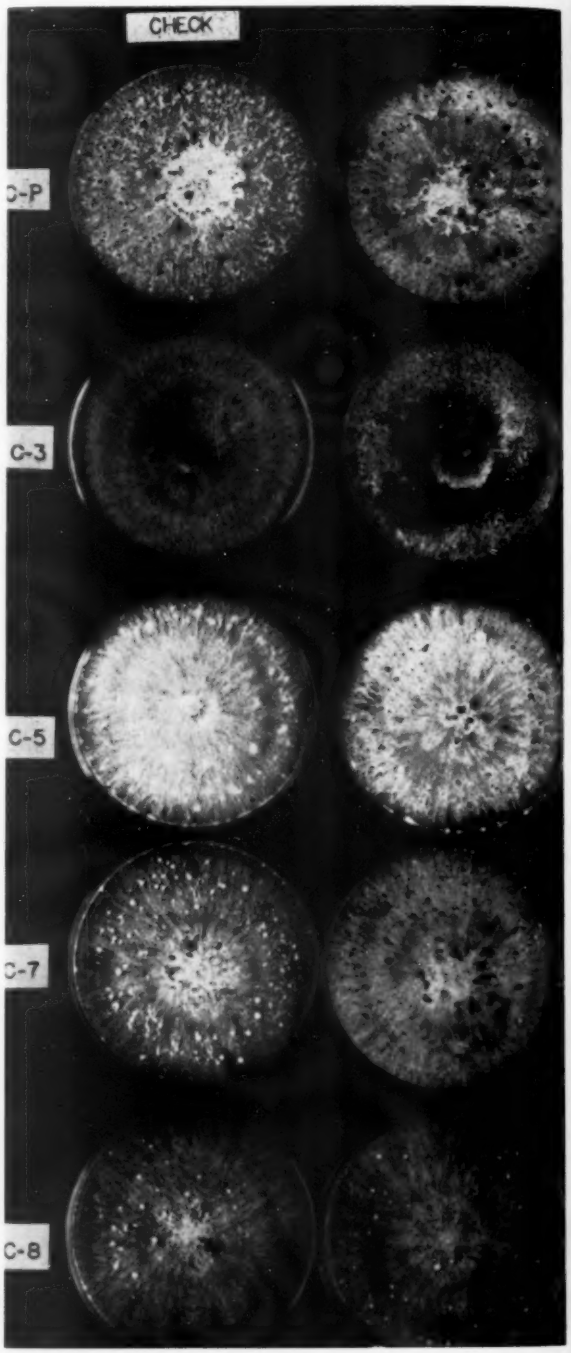

Fig. 1. Cultures of parent (C-P) and 4 mutants of *Diaporthe phaseolorum* var. *caulivora* 18 days after spermatization with conidia of parent type of the variety *sojae*. Note increased perithecia in mutants C-5, C-7, and C-8, and no perithecia in C-3.

the variety *sojae* as the mycelial parent and the variety *caulivora* as the paternal parent. No perithecia were produced in the crosses *inter se* of mutants C-2, C-3, C-5, C-6, C-6-1, C-7, C-8, and C-9-1 of the variety *caulivora* on soybean stems on water agar. Mutants C-5, C-6, C-6-1, C-7, and C-8 of the variety *caulivora* showed a marked increase in perithecial production when spermatized with conidia of the variety *sojae*. Particularly striking was the increased perithecial production of mutant C-7, see Fig. 1. Mutants C-2, C-3, and C-9-1 of the variety *caulivora* were not affected by spermatization with conidia of the parent type of the variety *sojae*. Self-sterile mutants C-2, C-6, and C-8 of the variety *caulivora* produced perithecia, and self-sterile mutants C-3, C-5, C-6-1, C-7, and C-9-1 failed to produce perithecia, when spermatized with the parent type of this variety.

Perithecia produced as a result of the crosses between mutant C-7 and the parent of variety *sojae* were long-beaked, single or in groups of two, and scattered (Fig. 2). Ascospores secured from the perithecia were bi-cellular, and in every way resembled those from the mycelial parent (*caulivora*). Moreover, perithecia formed by these crosses completely fit the description of the perfect stage of *D. phaseolorum* var. *sojae* in their characteristics, whereas they were different in their grouping and morphology from the variety *caulivora* (Fig. 3).

No perithecia were produced when mutants of the variety *sojae* were crossed *inter se* or with the parent type. No perithecia were produced when the variety *sojae* was the mycelial parent in crosses with mutants of the variety *caulivora*. However, fertile perithecia were obtained when parent types of the variety *caulivora* and the variety *sojae* were crossed with each other.

Analysis of the progeny from a cross of mutant C-7 and the parent type of the variety sojae.—Mutant C-7 of the variety *caulivora* was crossed with the parent type of the variety *sojae* of *Diaporthe phaseolorum*, and the progeny were analyzed by the single-spore method (13) on potato-dextrose agar (PDA). Mutant C-7 was tan and served as a marker in the analysis.

From 6 of the perithecia obtained in the F_1 generation, 115 single spores were isolated and incubated at 26°C, for 16 days, when perithecia were obtained from all cultures. Thirty-nine of the cultures obtained resembled neither parent, and 76 cultures resembled C-7. Three cultures of C-7 obtained in the F_2 generation were cultured and single-spored, and the progeny of the F_3 were studied. Segregation occurred among the 20 cultures obtained, and 2 cultures resembled C-7 in morphology and 18 cultures resembled neither parent.

From 21 perithecia of cultures that were different from either parent in the F_2 generation, 183 single spores were selected and incubated on PDA for 16 days at 26°C. Spores from 5 other perithecia sampled failed to germinate. As in the F_1, all cultures produced perithecia. Of the cultures in the F_3, 81 resembled mutant C-7 and 103 cultures resembled neither parent. The variety *sojae* was never recovered in segregates of either the F_2 or F_3 generations. Thus segregation after 2 successive generations resulted in the recovery of the maternal parent (*D. phaseolorum* var. *caulivora*) but not of the heterothallic parent (*D. phaseolorum* var. *sojae*). This was briefly reported previously (11).

Both parents (the variety *sojae* and mutant C-7 of the variety *caulivora*) were each single-spored for 3 successive generations with no segregation resulting in either parent.

Tests for stimulatory substances from spores of the variety sojae.—The possibility was investigated that conidia of *D. phaseolorum* var. *sojae* produced a substance that stimulated perithecia production by some mutants of *D. phaseolorum* var. *caulivora*.

Conidia from the variety *sojae* were suspended in

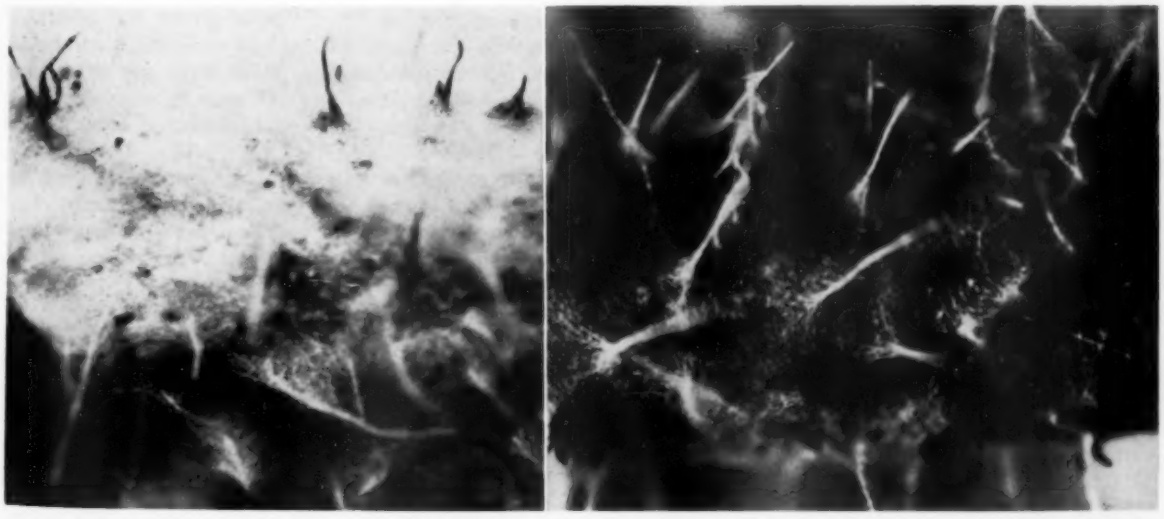

Fig. 2-3. 2) Long-beaked, scattered perithecia from a cross between mutant C-7 of the variety *caulivora* and the parent type of the variety *sojae* of *Diaporthe phaseolorum*. Cultures are 18 days old. **3)** Short-beaked, caespitose perithecia of *Diaporthe phaseolorum* var. *caulivora* on a soybean stem, 20 days after inoculation.

sterile water for 6 hours at room temperature. This suspension was filtered through a Bush positive-pressure filter apparatus with an ultrafine-porosity fritted glass disk. The resulting filtrate was sprayed over 4–5-day-old cultures of mutants of the variety *caulivora*. Controls were sprayed with sterile water only.

No increase was noted in numbers of perithecia produced by mutants C-5, C-7, C-8, and C-9-1 of *D. phaseolorum* var. *caulivora* after 18 days' incubation at room temperature.

Since the filtrate did not incite an increase in perithecia in these mutants, there apparently is no stimulatory substance produced by the conidia of the variety *sojae*. This would confirm the finding that a cross was produced between the parent type of this variety and mutants of the variety *caulivora*.

Nutrient studies.—Studies were made to determine whether or not self-sterility in mutants of *D. phaseolorum* var. *caulivora* was due to a nutrient deficiency, and to test the relative ability of the mutants and parent types of the varieties *sojae* and *caulivora* to grow on media other than PDA. In such studies the variety *sojae* and the variety *caulivora* were grown on minimal media amended with 15 carbon sources, 6 vitamin combinations, and 27 nitrogen sources.

Carbon sources were arabinose, cellobiose, dextrin, fructose, galactose, glucose, maltose, mannose, raffinose, rhamnose, starch, sorbose, sucrose, and xylose. Vitamins used were aminobenzoic acid, biotin, nicotinic acid, pyridoxine, and thiamine.

Nitrogen sources were alanine, arginine, aspartic acid, cysteine, cystine, glutamic acid, glycine, histidine, hydroxyproline, isoleucine, leucine, lysine, methionine, norleucine, phenylalanine, proline, serine, threonine, tryptophane, tyrosine, valine, urea, ammonium chloride, ammonium nitrate, ammonium nitrite, ammonium sulfate, sodium nitrate, and sodium nitrite.

Plates of media in the carbon and vitamin studies were inoculated with ascospores of the parent type and mutants C-7 and C-9-1 of the variety *caulivora*, with conidia of the parent type and mutants S-11, S-12, S-13, S-23, and S-27 of the variety *sojae*, and with hyphal tips of mutants C-3-1, C-4, C-5, C-6-1, and C-8 of the variety *caulivora*.

All cultures were incubated 10 days, and the growth of the colonies was determined by measuring colony diameter. These cultures were incubated an additional 10 days and the production of fruiting bodies was noted. Studies of nitrogen sources were done using tubes of media inoculated with mycelia of the mutants and parent types of the varieties *caulivora* and *sojae*.

Differences in growth were noted on the carbon sources tested, but sporulation was not affected by any of the carbon sources, nor were sterile mutants of the varieties *caulivora* and *sojae* stimulated to sporulate. Vitamin-amended media stimulated neither the mutants nor the parent types of the varieties *caulivora* and *sojae* to sporulate more or grow better than the controls.

Growth differences were evident between organisms grown on various nitrogen sources, but self-sterile mutants were not stimulated to sporulate. Evidence from the nutrient studies indicates that certain mutants of the varieties *caulivora* and *sojae* of *D. phaseolorum* can grow as well as the parent types on a wide range of media.

Self-sterility in mutants of the variety *caulivora* does not appear to be due to a deficiency in any of the nutrients studied.

Pathogenicity studies.—*D. phaseolorum* var. *sojae* has been described (10) as a mild parasite and a vigorous saprophyte, whereas *D. phaseolorum* var. *caulivora* has been reported (5, 7) to be a damaging parasite on soybeans, killing young plants by producing stem-girdling lesions. The variety *caulivora* can grow and sporulate on a wide variety of dead stems and leaves (5, 13).

The parent type of each variety had been isolated from Blackhawk soybeans. Therefore it was of interest to determine whether the mutants were also pathogenic on this host, and whether there were mutants that might be more pathogenic than the parent types.

For pathogenicity tests, 200 pots of autoclaved soil were seeded with 5 soybeans per pot. When 30 days old, the plants were inoculated at the lower leaf nodes by the toothpick method (2). Controls were inoculated with sterile toothpicks saturated with potato-dextrose broth.

Plants were inoculated with the parent type and mutants S-11, S-12, S-13, S-14, S-15, S-16, S-18, S-27, and S-30 of the variety *sojae*, and the parent type and mutants C-2, C-3-1, C-4, C-5, C-6-1, C-7, C-8, C-9-1, and C-10 of the variety *caulivora*. Plants in each of 4 pots were inoculated with each mutant and each parent type, and 20 pots of plants served as controls.

The controls were grown in the same greenhouse for another 24 days, and the plants were then checked for symptoms of pod and stem blight or stem canker.

The symptoms of stem canker were distinct, and the percentage of plants infected could be determined readily. The symptoms of pod and stem blight caused by *D. phaseolorum* var. *sojae* were not pronounced when compared to those of the controls; therefore infection was determined by reisolation of the organisms from the plants.

Mutant S-15 was isolated from more plants than was the parent type of variety *sojae*. All other mutants of the variety *sojae* tested were isolated from fewer plants than the parent type. Only mutant S-15 seemed as pathogenic as the parent type of the variety *sojae*, or more so.

All mutants of the variety *caulivora* were pathogenic, but none appeared more pathogenic than the parent. Mutants C-2, C-7, and C-8 were about as pathogenic as the parent on Blackhawk soybeans, whereas 3 other mutants were considerably less pathogenic. Also, some mutants of the variety *caulivora* produced symptoms of stem blight instead of stem canker, making impossible the identification of variety by symptoms.

DISCUSSION.—The parent types of *D. phaseolorum* var. *caulivora* and *D. phaseolorum* var. *sojae* appear

different in culture and react differently on soybeans. Though differences between parent types of the two varieties are distinct, these differences are less apparent among mutants of the two varieties.

The homothallic character of the variety *caulivora* does not appear to be constant. Frosheiser (5) and Hildebrand (7) both noted the production of pycnidia by certain isolates of this fungus. Certain mutants induced by irradiation became self-sterile, and behaved as heterothallic organisms, crossing with the parent type of the variety *sojae*.

The morphology of perithecia in the two varieties, used as a criterion for separating them, is also not constant. The parent type of the variety *caulivora* produces short-beaked perithecia in caespitose groups, and the parent type of the variety *sojae* produces scattered, single, long-beaked perithecia. Several mutants of the variety *caulivora*, however, produce single, scattered, long-beaked perithecia when crossed with the parent type of the variety *sojae*. Experimental evidence indicates that the variety *sojae* is the paternal parent only; therefore, the perithecia must have been produced by the mutants of the variety *caulivora*. Perithecial morphology thus does not seem to be a valid character to separate these two varieties.

Pathogenicity on soybeans has also been used for separating the two varieties. The parent type of the variety *caulivora* causes stem canker, and the parent type of the variety *sojae* causes pod and stem blight. Certain mutants of the variety *caulivora* did not cause stem canker in greenhouse tests, but produced symptoms of pod and stem blight on soybeans. It seems possible to the writers that similar mutants could exist in nature but would not be isolated, because most isolates are made from stem lesions. Such isolates would probably be classified as the variety *sojae*, which does not produce lesions, but are nevertheless pathogenic on soybeans.

If mutants were to occur and survive in nature, they could not be classified to a variety of *Diaporthe phaseolorum* on the basis of the criteria previously proposed to separate them. The characteristics of homo- and hetero-thallism, perithecial morphology, and relative pathogenicity appear to be definite in the parent types, but less so in their mutants. Differences that have been set for separating the two varieties are those that exist only between the parent types and do not include the variations from these types. Further complications are introduced in that hybrids of the two varieties can and probably do exist.

It is concluded by the writers that the 2 varieties described are not distinctly different, based on morphology of perithecia and pathogenicity on soybean, when the range of variation of each is considered.

DEPARTMENT OF PLANT PATHOLOGY AND BOTANY
INSTITUTE OF AGRICULTURE
UNIVERSITY OF MINNESOTA
ST. PAUL 1, MINNESOTA

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COMPARATIVE INHIBITION OF VIRUS MULTIPLICATION BY CERTAIN TYPES OF CHEMICALS[1, 2]

R. C. Lindner, Hugh C. Kirkpatrick, and T. E. Weeks

SUMMARY

Two hundred and thirty-three chemicals were screened for their ability to inhibit TMV (tobacco mosaic virus) multiplication in cucumber cotyledons. More limited screening was done with stone fruit ring spot virus. Twenty-seven of the more effective chemicals were tested in a range of concentrations; the concentration giving 50% inhibition of TMV was used for comparison. Seventeen of the 27 chemicals have not previously been reported as active against plant viruses. The 15 chemicals effective at 1m*M* or less included 2 purine or pyrimidine derivatives (thiouracil and 2,6,8-trichloropurine); 6 dyes (methylene blue, brilliant green, crystal violet, malachite green, eosin B, and eosin Y); 1 inorganic salt (sodium selenate); 4 antibiotics (chloramphenicol, noformicin, cytovirin, and actidione); and 2 folic acid derivatives (A-methopterin and A-denopterin). Acti-dione was the most active chemical tested, giving 50% inhibition of TMV at only 2 ppm. Surfactants were shown to be good surface inhibitors of infection but of no systemic value. The cationic types were more effective than anionic or nonionic. Lauryldimethylbenzylammonium chloride (Zephiran), the most effective surfactant, gave 50% inhibition of TMV lesions at 40 ppm.

INTRODUCTION.—The literature includes a number of reports on the effectiveness of various chemicals in inhibiting virus multiplication in plants. Recent reviews by Bawden (1) and Matthews and Smith (7) cover the subject adequately, but there have been no comprehensive reports on the comparative molar activity of different chemicals. This paper reports the results of extended screening trials carried out over the past 6 years.

MATERIALS AND METHODS.—To facilitate testing a large number of different chemicals, the study was limited to 2 different plant viruses. Most of the work was done with the relatively stable tobacco mosaic virus (TMV). Studies were also made with a very unstable virus, stone fruit ring spot (RSV), mechanically transferable to cucumber, which has been kept continuously in the greenhouse for 7 years by weekly passages through cucumber seedlings. RSV was tentatively designated PLMV in previous publications (2, 3, 4) until its identity was firmly established.

All tests were confined to cucumber seedlings in order to maintain a common host. RSV is systemic in cucumber, and TMV produces a local starch lesion. Chicago Pickling cucumber seeds (*Cucumis sativus* L.) were sown, 2 to a 2½-in. pot, and thinned to 1 good seedlings. Seeds, germinated in a greenhouse at about 21°C, were usually ready for inoculation 10 days after planting. Unless otherwise indicated all tests were carried out at about 21°C.

TMV was purified from heat-clarified, homogenized, infected tobacco leaves by repeated differential centrifugation. This stock culture was kept in concentrated form in distilled water at 4°C and used in all of the work reported here. Inoculation was made in the afternoon with the stock diluted to 100 or 200 ppb TMV, with 5% 600-mesh carborundum in the inoculum, following optimum procedures for infection previously reported (5). A minimum of 20 plants were used per treatment, giving 40 individual cotyledon replicates. It was not possible to use paired cotyledons, because, in preliminary trials, some materials were translocated from the treated to the untreated cotyledon on the same plant. A minimum difference of 30% was required for significance.

RSV inoculum was prepared from freshly homogenized infected cucumber cotyledons. One part by weight of tissue was homogenized with 9 parts of buffer. The buffer was composed of 0.007*M* neutral potassium phosphate, 0.01*M* sodium diethylbarbiturate, 0.007*M* neutral potassium ethylenediamine tetraacetate, and 0.01*M* cysteine-HCl. The buffer was ad-

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TABLE 1.—*Relative inhibition of TMV lesions by surfactants applied at 500 ppm immediately after inoculation*

Surfactant	Type	Per cent inhibition
Lauryldimethylbenzylammonium chloride (Zephiran)	Cationic	94
Cetyltrimethylammonium bromide	Cationic	88
Cetylpyridinium chloride	Cationic	68
Diisobutyl-*p*-cresoxyethyldimethyl-benzyl-ammonium chloride	Cationic	64
Bis(2-ethyl hexyl) sodium sulfosuccinate (Aerosol OT)	Anionic	60
Sodium lauryl sulfate	Anionic	60
Commercial A	Anionic	54
Commercial B	Anionic	53
Commercial C	Anionic	50
Commercial D	Anionic	9
Commercial E	Nonionic	43
Commercial F	Nonionic	42
Commercial G	Nonionic	34
Commercial H	Nonionic	30
Polyoxyethylene sorbitan monolaurate (Tween 20)	Nonionic	7
Commercial I	Nonionic	5

justed to pH 7.8 by adding 1*M* KOH (4).

All treatments were applied 24 hours after inoculation. For TMV, test solutions were brushed on. For proper wetting of the cotyledon surface, an appropriate surfactant was incorporated in the test solution. Controls, with surfactant alone, were included in each test. For RSV, test solutions were vacuum infiltrated into the cotyledons (3). Plants were inverted over beakers in a desiccator with cotyledons immersed in the test solution. A vacuum of about 127 mm of mercury was applied for 3 minutes. Upon release of the vacuum, the intercellular spaces were filled with the test solution.

TMV was assayed by local-lesion counts. Six or 7 days after inoculation the cotyledons were harvested and stored in the dark in a moist chamber for 24 hours to remove excess starch. Chlorophyll was removed by heating with 70% ethanol in an 80°C water bath. Starch lesions were developed by placing the cleared cotyledons in an IKI–lactic-acid mixture for at least 15 minutes. The stock IKI mixture consisted of 2% iodine and 6% KI. One part of this stock was mixed with about 20 parts of lactic acid for staining. Lesions were counted with the aid of a low-power microscope (12.5×).

RSV was assayed spectrophotometrically following procedures previously reported (2).

RESULTS.—*Effect of surfactants.*—It has been shown (5) that surfactants inhibit virus infection if applied shortly after inoculation. Even 24 hours after inoculation, surfactants can inhibit a relatively high percentage of infection sites. Since a surfactant was desired in the chemical treatments of TMV for better coverage of the test chemical, a number of surfactants were tested for their ability to inhibit TMV infection. For comparative purposes, all surfactants were applied by brush at 500 ppm immediately after inoculation. Cationic types of surfactants were, in general, the most effective, and nonionic surfactants were the least effective (Table 1).

The effect of concentration of 3 representative types of surfactants, applied immediately after inoculation, on TMV lesions is shown in Fig. 1. Zephiran, the most active of the surfactants, shows promise of being a good surface-sterilant for viruses. Because of its effectiveness in inhibiting lesions, it was not used in treatments with other chemicals. Either Aerosol OT or Tween 20 were the surfactants chosen, depending on the ionic nature of the chemical in question.

Purines and pyrimidines.—For TMV screening tests, various purines and pyrimidines were dissolved at 300 ppm in 0.01*M* sodium carbonate and 1000 ppm Aerosol OT. For RSV tests, a concentration of 0.001*M* purine or pyrimidine in 0.001*M* NaOH was used because the infiltration procedure allows better penetration of the test chemical. The results for 31 purines are shown in Table 2. 6-methylpurine, 2,6,8-trichloropurine, and 8-azaguanine were effective against both viruses. 6-methylpurine, however, was too phytotoxic to be of use. RSV appeared to be more sensitive than TMV (or the test method was more sensitive) because, in addition to the above purines, 2,6-diaminopurine, 2,6-bis-diethylaminopurine, 8-aza-2,6-diaminopurine, and 2-mercaptopurine were effective in RSV inhibition.

The results of tests with 55 pyrimidines are shown in Table 3. It will be noted that only thiouracil and 2,4,6,8-tetrachloropyrimidine have enough activity to be of possible use for either virus.

Inorganic salts were screened at 500 ppm in 1000 ppm Tween 20 against TMV. None of the salts were highly active in TMV inhibition. Copper sulfate, mercuric chloride, silver nitrate, sodium arsenate, and sodium selenate gave moderate inhibition. The last two salts were phytotoxic at 500 ppm. The following salts gave no significant TMV inhibition: lithium nitrate, sodium fluoride, aluminum chloride, silicic acid, titanium trichloride, ammonium vanadate, sodium chro-

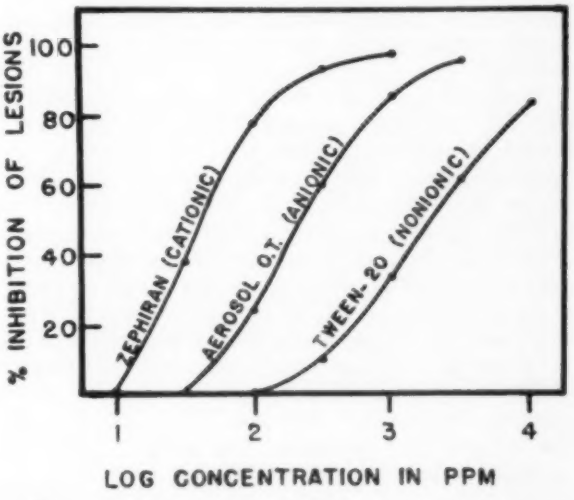

Fig. 1. Effect of concentration of 3 representative surfactants in inhibiting TMV lesions when applied immediately after inoculation.

TABLE 2.—*Effect of various purines in inhibiting multiplication of TMV and RSV*

Purine[a]	Substituted position						Virus inhibition[b]	
	1	2	3	6	7	8	TMV	RSV
Adenine				NH_2			–	–
Guanine		NH_2		O			–	–
Hypoxanthine				O			–	–
Xanthine		O		O			–	–
Isoguanine		O		NH_2			–	–
Uric acid		OH		OH		O	–	–
2-aminopurine		NH_2					–	–
2,6-diaminopurine		NH_2		NH_2			–	+
Theobromine		O	CH_3	O	CH_3		–	–
Caffeine	CH_3	O	CH_3	O	CH_3		–	–
Ethyltheobromine		O	C_2H_5	O	C_2H_5		–	–
Propyltheobromine		O	C_3H_7	O	C_3H_7		–	–
6-methylpurine				CH_3			+[c]	+[c]
2,6-bisdiethylaminopurine		$<^{NH_2}_{C_2H_5}$		$<^{NH_2}_{C_2H_5}$			–	+
8-chloroxanthine		O		O		Cl	–	–
6-chloropurine				Cl			–	–
2,6,8-trichloropurine		Cl		Cl		Cl	++	++
2-thioadenine		S		NH_2			–	–
6-thioxanthine		O		S			–	–
2-thio-6-oxypurine		S		O			–	–
2-mercaptopurine		S					–	+
6-mercaptopurine				S			–	–
2,8-dithio-6-oxypurine		S		O		S	–	–
2-thio-6-methylpurine		S		CH_3			–	–
8-azaguanine		NH_2		O		N[d]	–	++
8-azaadenine				NH_2		N	–	–
8-azaxanthine		O		O		N	–	–
8-aza-2,6-diaminopurine		NH_2		NH_2		N	–	++
8-aza-2-thioguanine		S		O		N	–	–
1,3-dimethyl-8-azaxanthine	CH_3	O	CH_3	O		N	–	–
Kinetin				$-NHC_5H_5O$			–	

[a] Purine tested at 300 ppm in 0.01*M* Na_2CO_3 and 1000 ppm Aerosol OT for TMV and at 0.001*M* in 0.001*N* NaOH for RSV.

[b] – = no significant inhibition; + = 30 to 60% inhibition; ++ = over 60% inhibition.

[c] 6-methylpurine highly phytotoxic. Concentration reduced to 5×10^{-4} *M*.

[d] The symbol N indicates nitrogen replaces carbon in the ring.

mate, chromium ammonium sulfate, manganous chloride, ferric chloride, cobaltous nitrate, zinc sulfate, strontium chloride, zirconium nitrate, ammonium molybdate, stannous chloride, potassium iodide, potassium iodate, barium chloride, silicotungstic acid, lead nitrate, ceric sulfate, thorium nitrate, and uranium acetate.

Twelve of the 35 dyes tested showed some inhibition of TMV. A dye concentration of 500 ppm in 1000 ppm Tween 20 was used. Eosin Y and eosin B gave marked TMV inhibition. The following dyes were moderately active: acridine orange, acridine red, pyronine B, neutral red, toluidine blue, methylene blue, thionine, malachite green, brilliant green, and crystal violet. Acridine orange and acridine red, however, were too phytotoxic to be of use. No activity was shown by picric acid, martius yellow, acriflavine, carmine, alizarin red S, purpurin, celestine blue, pyronine G, fluorescein, dichlorofluorescein, hematoxylin, safranin, methyl orange, titan yellow, basic fuchsin, methyl violet, methyl green, aniline blue, light green S. F., aurin, phenolphthalein, bromthymol blue, and auramine.

Antibiotics were usually screened at 100 ppm in Tween 20 against TMV. D- or DL-chloromycetin, noformicin, cytovirin and acti-dione markedly inhibited TMV. Nicetin gave moderate inhibition and phytoactin and phytostreptin gave slight inhibition; whereas aureomycin, penicillin, terramycin, streptomycin, tetracycline, neomycin, fungichromin, polymixin and griesofulvin gave no inhibition. Four derivatives of actidione were compared against the parent material, but none were so active. Acti-dione S and T gave marked TMV inhibition at 100 ppm, whereas acti-dione M gave moderate inhibition and acti-dione AA gave no inhibition.

Four benzimidazole derivatives were tested at 300 ppm in 1000 ppm sodium carbonate plus 1000 ppm Tween 20. None of these were effective against TMV. They included benzimidazole, 5-methylbenzimidazole, 2-benzimidazolethiol, and 4,5,6-(5,6,7,)-trichloro-1-(D-D-ribofuranosyl)-benzimidazole. The latter compound has been reported to give 75% inhibition of polio virus multiplication at only 4.2 ppm (7).

Of 4 folic acid derivatives tested against TMV, 2 proved highly active. A-denopterin and A-methopterin both gave marked inhibition of TMV, whereas 9-methyl folic acid and N^{10}-methylpteroic acid were ineffective.

None of 8 phenols tested inhibited TMV. These included ortho-, meta-, and para-nitrophenol, 2,4-dinitrophenol, 2,6-dinitro-4-chlorophenol, 2,4,6-trinitrophenol,

dipicrylamine, and 2,5-bis-ethyleneimino hydroquinone.

None of the 4 carbamates inhibited TMV. These included glycine, biuret, furfuryl-*N*-phenylcarbamate, and furfuryl-*N*-(5-chloro-2-methylphenyl) carbamate.

Procaine-HCl moderately inhibited TMV, and the following unclassified materials were ineffective: asparagine, ammonium sulfamate, hydrazine, sulfanilimide, m-phenylenediamine, *N,N*,-dimethyl-*p*-nitrosoaniline, sodium dichloroisobutyrate, tetramethylthiouram disulfide, benzidine HCl, 1-chloro-2,4-dinitrobenzene, hexamethylenetetramine, betabion, ascorbic acid, dithiodiglycol, furfural, nitrofurazone, DL-p-fluorphenylalanine, 8-hydroxyquinoline, 4-aminotriazole, merthiolate, naphthalene acetic acid, benzothiazole-2-oxyacetic acid, triphenyltetrazolium chloride, colchicine, and 3,5-dimethyltetrahydro-1,3,5-2*H*-thiadiazine-2-thione.

TABLE 3.—*Effect of various pyrimidines in inhibiting multiplication of TMV and RSV*

Pyrimidine[a]	Substituted position						Virus inhibition[b]	
	1	2	3	4	5	6	TMV	RSV
Uracil		O		O			–	–
Cytosine		O		NH_2			–	–
Thymine		O		O	CH_3		–	–
Dihydrothymine		OH		OH	CH_3		–	–
Barbituric acid		O		O		O	–	–
Orotic acid		O		COOH		O	–	–
6-aminocytosine		O		NH_2		NH_2	–	–
2,4-dihydroxy-6-aminopyrimidine		OH		OH		NH_2	–	–
2,6-dihydroxy-4-aminopyrimidine		OH		NH_2		OH	–	–
1,3-dimethyluracil	CH_3	O	CH_3	O			–	–
2-amino-4-methylpyrimidine		NH_2		CH_3			–	–
Methyl ureido propylene urea		O	$NHCONH_2$	CH_3			–	–
5-aminouracil		O		O	NH_2		–	–
5-bromuracil		O		O	Br		–	–
5-nitrouracil		O		O	NO_2		–	–
2-amino-4-methoxypyrimidine		NH_2		OCH_3			–	–
5-carbethoxycytosine		O		NH_2	$COOC_2H_5$		–	–
Diazouracil		O		O	N≡N		–	–
Isocytosine		NH_2		O			–	–
2-aminopyrimidine		NH_2					–	–
4,6-dihydroxypyrimidine				OH		OH	–	–
2-amino-4,6-dioxypyrimidine		NH_2		O		O	–	–
2,4-diamino-6-oxypyrimidine		NH_2		NH_2		O	–	–
2,4,6-triaminopyrimidine		NH_2		NH_2		NH_2	–	–
3-isobutyl-5-carbethoxyuracil		O	C_4H_9	O	$COOC_2H_5$		–	–
3-ethyl-5-cyanocytosine		O	C_2H_5	NH_2	CN		–	–
Barbital		O		O	$(C_2H_5)_2$	O	–	–
Alloxan		O		O	O	O	–	–
2,5-diamino-4,6-dioxypyrimidine		NH_2		O	NH_2	O	–	–
2,4,5-triamino-6-hydroxypyrimidine		NH_2		NH_2	NH_2	OH	–	–
4,5,6-triaminopyrimidine				NH_2	NH_2	NH_2	–	–
2,4,5,6-tetraaminopyrimidine		NH_2		NH_2	NH_2	NH_2	–	–
2-thiouracil		S		O			++	++
2-thiocytosine		S		NH_2			–	–
5-thiouracil		O		O	S		–	–
2,4-dithiopyrimidine		S		S			–	–
2-amino-4-thiopyrimidine		NH_2		S			–	–
5-methylthiouracil		S		O	CH_3		–	+
5-carboxythiouracil		S		O	COOH		+	–
6-methylthiouracil		S		O		CH_3	–	–
6-propylthiouracil		S		O		C_3H_7	+	–
6-aminothiocytosine		S		NH_2		NH_2	–	–
2-thiobarbituric acid		S		O		O	–	–
3-allyl-5-carbethoxythiouracil		S	$CH=CH_2$	O	$COOC_2H_5$		+	+
3-methyl-5-carbethoxythiouracil		S	CH_3	O	$COOC_2H_5$		–	–
2-thio-4,4,6-trimethyldihydropyrimidine		S		$(CH_3)_2$		CH_3	–	–
3-butyl-5-cyano-6-methyl-thiouracil		S	C_4H_9	O	CN	CH_3	–	–
2-chloro-4-amino-5-hitropyrimidine		Cl		NH_2	NO_2		–	–
2,4-dichloropyrimidine		Cl		Cl			–	–
2-amino-4-chloropyrimidine		NH_2		Cl			–	–
2,4-dichloro-6-methylpyrimidine		Cl		Cl		CH_3	–	–
4,6-dichloropyrimidine				Cl		Cl	–	–
2,6-dichloropyrimidine		Cl				Cl	–	+
2,4,6-trichloropyrimidine		Cl		Cl		Cl	–	–
2,4,5,6-tetrachloropyrimidine		Cl		Cl	Cl	Cl	++	++

[a] Pyrimidine concentration 300 ppm in 0.01*M* Na_2CO_3 plus 1000 ppm Aerosol OT for TMV, and 0.001*M* in 0.001*N* NaOH for RSV.

[b] – signifies no significant inhibition; + signifies 30–60% inhibition; ++ signifies over 60% inhibition.

TABLE 4.—*Comparative inhibition of TMV lesions by the most active antiviral chemicals*

Type	Chemical	Concentration required to give 50% inhibition of TMV lesions μM
Purines and pyrimidines	8-azaguanine	8000
	8-aza-2-thioguanine	4100
	2,4,5,6-tetrachloro pyrimidine	1400
	Thiouracil	1000
	2,6,8-trichloropurine	540
Dyes	Toluidine blue	2800
	Neutral red	2200
	Pyronin B	2000
	Thionin	1700
	Methylene blue	1100
	Brilliant green	330
	Crystal violet	290
	Malachite green	270
	Eosin B	16
	Eosin Y	12
Inorganics	Mercuric chloride	4400
	Cupric sulfate	4400
	Silver nitrate	2600
	Sodium arsenate	2100
	Sodium selenate	810
Antibiotics	D-chloromycetin	620
	Noformicin	270
	Cytovirin	45
	Acti-dione	7
Folic acid derivatives	A-methopterin	220
	A-denopterin	43
Not classified	Procaine hydrochloride	2200

Any material that gave more than 30% inhibition to TMV in screening tests was tested over a range of dilutions. The concentration that gave 50% inhibition of TMV lesions was chosen as a convenient comparative point. The results are shown in Table 4. The antiviral activity of some of the antibiotics, dyes, and folic acid derivatives were outstanding. Acti-dione was the most active of any material tested giving 50% inhibition of TMV lesions at only 2 ppm.

DISCUSSION.—Relatively few of the substituted purines and pyrimidines inhibit virus multiplication. It was hoped that, by testing a series of related purines and pyrimidines, some correlation between structure and activity would be noted. This did not prove to be the case. All substitutes in the 3, 4, 5, or 6 positions of thiouracil decreased activity, and no other thio-substituted pyrimidine had activity. The chlorinated pyrimidines had no activity except for a slight amount with the 2,6 and a moderate amount with the 2,4,5,6 substituted compounds. One unknown factor in this work is the ability of the host plant to modify the test chemical, and this factor may have been important. There is little information available on this point, although it has been reported (6) that 8-azaguanine is rapidly deaminated in young pea plants in the dark.

A small change in the test molecule apparently can make a large difference in its ability to block virus multiplication. This is well illustrated with the triphenylmethane dyes. Malachite green, brilliant green and crystal violet all showed moderate activity, but the closely related methyl violet and methyl green were inactive. The reason is not clear.

The following antiviral agents shown in Table 4 have not been previously reported as active against plant viruses: 8-aza-2-thioguanine, 2,4,5,6-tetrachloropyrimidine, 2,6,8-trichloropurine, toluidine blue, neutral red, pyronin B, thionin, brilliant green, eosin B, eosin Y, mercuric chloride, sodium arsenate, sodium selenate, acti-dione, A-methopterin, A-denopterin, and procaine HCl.

It is difficult to compare the results of other workers on those chemicals that have previously been reported to be active because: 1) there are few quantitative data available, 2) different host-virus systems are involved, 3) different methods of application are used, and 4) different test methods are used.

The mechanism of action of these various materials in blocking TMV multiplication is probably diverse. The action of some of the materials may be exclusively on the host plant. It should be pointed out that, at the point of 50% inhibition, none of the materials in Table 4 were markedly phytotoxic. The inorganic materials, however, were on the borderline. At higher concentrations they were usually toxic to the test plants.

The substituted purines and pyrimidines on an equal molar basis are not so active as some of the dyes, antibiotics, or folic-acid derivatives. In a search for more active chemicals, the antibiotics would seem to be a promising field to explore.

DEPARTMENT OF PLANT PATHOLOGY
STATE COLLEGE OF WASHINGTON
TREE FRUIT EXPERIMENT STATION
WENATCHEE, WASHINGTON
AND
CROPS RESEARCH DIVISION
AGRICULTURAL RESEARCH SERVICE
U. S. DEPARTMENT OF AGRICULTURE
WENATCHEE, WASHINGTON

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COCHLIOBOLUS CARBONUM, THE PERFECT STAGE OF HELMINTHOSPORIUM CARBONUM[1]

R. R. Nelson[2]

SUMMARY

The perfect stage of *Helminthosporium carbonum* was produced in culture by pairing compatible conidial isolates. Ascocarp production was most satisfactory when compatible isolates were paired on opposite sides of a section of sterile corn leaf in a Petri plate containing Sach's nutrient agar adjusted to pH 4. At 24°C, immature perithecia appeared in 5–6 days and most of the functional asci contained mature spores by 22 days. The perfect stage is described as *Cochliobolus carbonum*. Conidial and ascospore isolates were about equally divided into 2 mating groups, although none of the isolates were completely intergroup fertile. The inconsistent behavior of paired compatible strains suggests that a number of genes are involved in the completion of sexual reproduction. The fact that ascocarps of this and other species are produced in agar culture only on solid objects, usually plant parts, suggests a physical requirement necessary for ascocarp initiation and development.

Studies of perfect stages of plant-parasitic species of *Helminthosporium* are important from both a mycological and a pathological standpoint. Further information on the relationships among fungus species of diverse or related natural groups should provide a better understanding of the evolution of the fungi. It is recognized also that a thorough knowledge of the genetic potentialities of plant pathogens would be helpful in breeding for disease resistance. The perfect stage of *Helminthosporium carbonum* Ullstrup, causal agent of a leaf spot and ear rot of corn, was recently obtained in paired cultures of conidial isolates. This paper reports the methods used in obtaining ascocarps of the fungus in paired cultures. The morphology and taxonomy of the species is discussed, and evidence is presented of its heterothallic nature.

MATERIALS AND METHODS.—The 18 monoconidial isolates used were obtained from leaf lesions and infected ears of corn from North Carolina, Virginia, Georgia, Missouri, Texas, Indiana, Maryland, and Nicaragua, Central America. Twenty-five monoascosporic cultures isolated from ascocarps produced in culture were also studied.

Mature perithecia were produced when small pieces of mycelium of compatible isolates were placed on opposite sides of a section of sterile corn leaf placed in the center of a Petri plate containing Sach's nutrient agar. The sections of corn leaf, about $1\frac{1}{2}\times\frac{1}{2}$ in., were sterilized by autoclaving for 1 hour. Fertile ascocarps were also formed when isolates were paired on opposite sides of sterile barley grains, but fewer perithecia were produced and these usually contained fewer asci and ascospores. Several matings involving known compatible isolates on agar media without corn leaf tissue or barley grains produced no fertile perithecia.

Perithecial production was most abundant when the medium was adjusted to pH 4. Additional studies indicated that light was not a factor in sexual reproduction.

Development of perithecia was most consistent and abundant when inoculated plates were kept continually at 24°C. Ascocarp production was sparse at 20 and 30°C. Under optium conditions, immature perithecia appeared in 5–6 days and developed immature asci by 10–12 days. A small proportion of the asci contained mature ascospores in 14 days, and most of the functional asci contained mature spores by 22 days.

Morphology and taxonomy.—Mature perithecia (Fig. 5) are black and ellipsoidal to globose. They measure 355–550 μ in height and 320–430 μ in diameter. Long brown setae are produced over the upper fourth of the perithecium. Conidiophores and conidia develop frequently on the perithecium. A well-defined ostiolate beak, subconical to paraboloid and 60–200 μ long, is usually produced. A mass of hyaline cells frequently covers the apex of the beak.

The locule is filled with a mass of hyaline, filamentous pseudoparaphyses. Asci (Fig. 1, 2, 3) are produced among the pseudoparaphyses and arise from the base of the locule. The asci are cylindrical or clavate, and straight or slightly curved, with a short stipe. Asci appear to be unitunicate, for there is no evidence of an extensible inner wall. They measure 162–257×18.2–27.4 μ (mean 196.6×22.6 μ). Asci contain 1–8 ascospores coiled in a close helix. Asco-

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[2] Plant Pathologist, Crops Research Division, ARS, U. S. D. A., Raleigh, North Carolina.

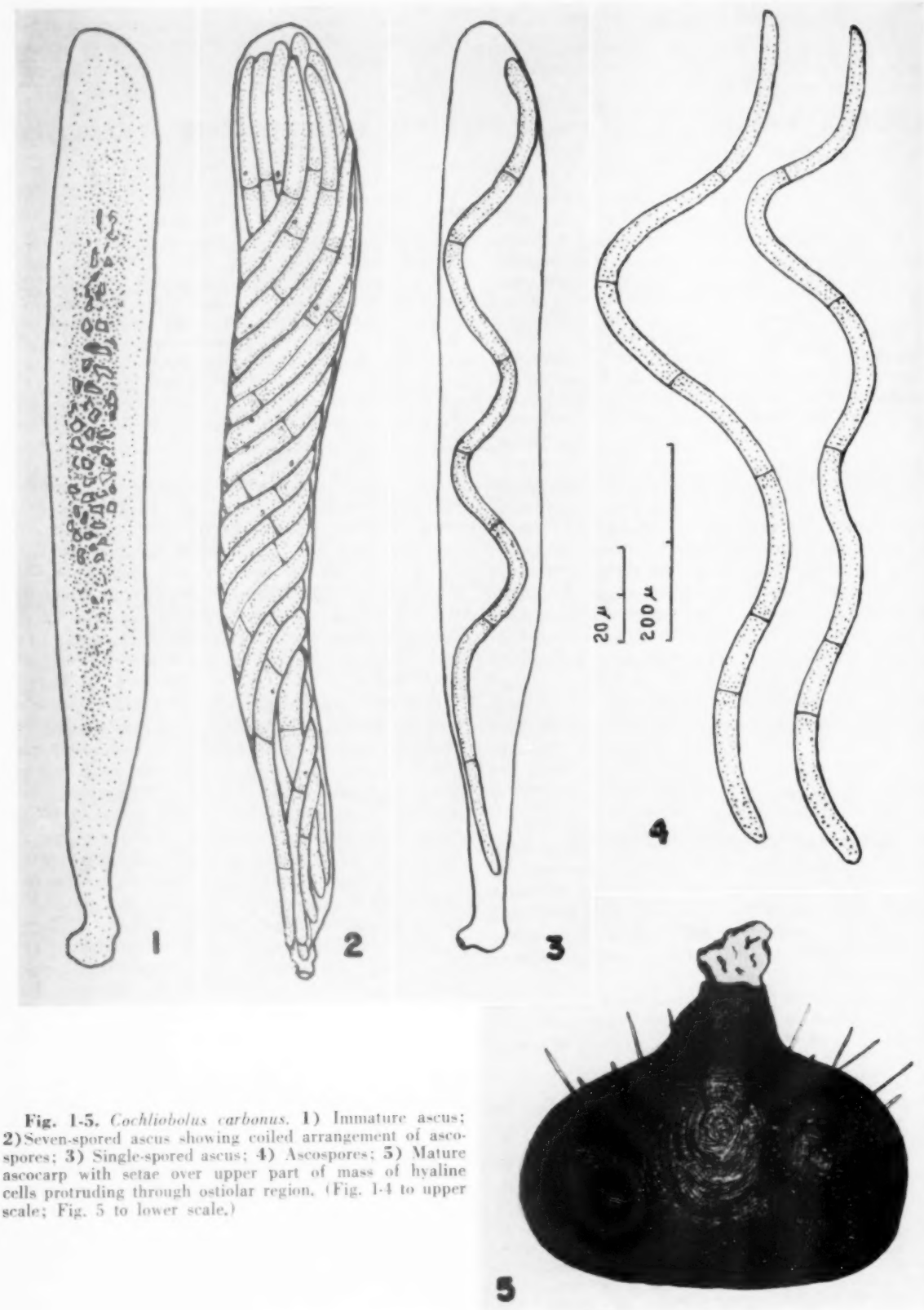

Fig. 1-5. *Cochliobolus carbonus.* **1)** Immature ascus; **2)**Seven-spored ascus showing coiled arrangement of ascospores; **3)** Single-spored ascus; **4)** Ascospores; **5)** Mature ascocarp with setae over upper part of mass of hyaline cells protruding through ostiolar region. (Fig. 1-4 to upper scale; Fig. 5 to lower scale.)

spores are discharged by the splitting of the upper part of the ascus wall.

The ascospores (Fig. 2, 3, 4) are filiform or flagelliform, and somewhat tapered at the extremities. Mature ascospores are typically hyaline, with 5–9 septa, and measure 182.0–306.4×6.4–9.6 μ (245.4×7.8 μ). Following discharge, the ascospores are often surrounded by a thin mucous envelope. Germination is either terminal or lateral.

The fungus belongs in the genus *Cochliobolus*, erected by Drechsler (1) to include species formerly considered in *Ophiobolus*, but differing in having filiform ascospores in helicoid arrangement and *Helminthosporium* conidial stages. The proper taxonomic position of *Cochliobolus* is questionable, but this paper's purpose is not to resolve existing confusion or add to it. Considering *H. carbonum* to be a valid and distinct conidial stage, and in view of the fact that no perfect stage has hitherto been associated with *H. carbonum*, the fungus is described as a new species of *Cochliobolus*, as follows:

Cochliobolus carbonum sp. nov.—Ascomata nigra, ellipsoideo-globosa, ostiolata, 355–550 μ alta, 320-430 μ in diametro, per quadrantem superiorem setis longis brunneis tecta, saepe conidiophora et conidia ferentia; rostrum ostiolarem definitum, subconicum, usque paraboloideum, 60–200 μ longum, ad apicem saepe massa cellularum hyalinarum tectum; loculus massa pseudoparaphysium hyalinarum filamentosarum et ascorum impletus; asci cylindricoclavati, recti vel subcurvati, breve stipitati, adparenter unitunicati, 162–257 × 18.2–27.4 μ (mediani 196.6 × 22.6 μ); parte superiori fissente et ascosporas ejiciente; ascosporae 1–8, in helice stricta circinnati, filiformes vel flagelliformes, ad apices attenuatae, hyalinae, 5–9-septatae, 182.0–306.4 × 6.4–9.6 μ (245.4 × 7.8 μ), e tegumento tenui mucoso circumdatae, terminaliter vel lateraliter germinantes.

Type specimens have been deposited in the National Fungus Collections, Beltsville, Maryland, and the Herbarium of the Royal Botanic Garden, Kew.

Heterothallism.—Studies with selfed and paired cultures of conidial and ascosporic isolates of *C. carbonum* have indicated that the species is heterothallic. Mature ascocarps were produced only when monoascosporic or monoconidial isolates were grown in pairs. Single monoascosporic or monoconidial isolates produced only protoperithecia, or none at all.

Mature ascocarps were obtained initially in a mating of conidial isolates 101 3a and 101 7a. For study of the mating reactions of the other 16 monoconidial isolates and the 25 monoascospore isolates obtained from a cross of 101 3a and 101 7a, isolate 101 3a was arbitrarily designated "plus" and isolate 101 7a "minus." The 16 monoconidial isolates were mated to 101 3a and 101 7a and were also mated in all possible pairs. Mating reactions of only 8 of the 18 conidial isolates were identified as a result of 5 complete mating tests. Four of the remaining crosses were fertile in 2 tests, and 2 were fertile in only one test.

The 25 ascospores obtained from a cross of 101 3a × 101 7a were backmated in 4 separate tests to 101 3a and 101 7a. As a result, the mating reactions of 14 of the isolates were identified. Again the matings were inconsistent, in that only 3 ascospore isolates were fertile with the compatible parental strain in all 4 of the tests.

By using other conidial isolates and selected ascospore isolates as additional testers, the mating reactions of 16 conidial isolates and all 25 of the ascospore isolates were identified. Of the conidial isolates, 5 were "plus" strains, 11 were "minus" strains, and 2 were not fertile with testers of either group. Fourteen of the monoascosporic isolates were "plus" strains and 11 were "minus" strains.

It is significant that only 12 of a possible 55 intergroup crosses between "plus" and "minus" conidial isolates have been fertile. It is indicative of a genetic difference in mating reaction similar to that described in *Cochliobolus heterostrophus* Drechs. (*Helminthosporium maydis* Nisik. & Miyake) (6) and *Trichometasphaeria turcica* Lutt. (*Helminthosporium turcicum* Pass.) (3, 5).

Although data are not now sufficient to determine the genetic basis for mating reaction, these studies demonstrate the *C. carbonum* is heterothallic in that conidial and ascosporic isolates of the fungus are self-sterile and cross-fertile.

Discussion.—When isolates of *H. carbonum* were first studied by Ullstrup (9), they were considered to be specialized races of *H. maydis*. In later studies, Ullstrup (10) critically compared the conidial morphology, spore color, and mean spore lengths of the isolates and noted differences between the isolates sufficient to warrant species separation. Discovery of the perfect stage of *H. carbonum* has provided an additional criterion for separating the species. The fact that isolates of each species are interfertile and that all attempts to cross isolates of *H. carbonum* and *H. maydis* have been unsuccessful further demonstrates genetic differences between the 2 species. However, similarities in the morphology of the conidial and perfect stages of the 2 species, suggesting a close natural relationship, encourage further attempts at interspecific crosses with a more diverse genetic background of the 2 species.

In recent years, monoascosporic isolates of at least 3 species of *Cochliobolus* have been studied in culture. All the species, *C. nodulosus* Luttrell (2), *C. sativus* (Ito & Kurib.) Drechs. ex Dastur (7, 8), and *C. heterostrophus* (4), have been shown to be heterothallic.

In paired cultures of the 3 species, as well as *C. carbonum*, ascocarps developed only on a solid object, usually a sterilized plant part, placed on or partially embedded in the agar substrate. Barley grains, corn grains, barley straw, and fragments of corn leaves were used most extensively, but results were similar with other plant parts. Mature ascocarps of *C. heterostrophus*, for example, were produced on leaf fragments of 16 diverse plant species. Sparse ascocarp production was also obtained when pieces of filter paper were placed on the agar substrate. These results suggest that the solid object provided a physical requirement rather than a nutritional one. It is possible

that sterilized plant parts contribute minute quantities of 1 or more nutrients, but the nutritional contribution of filter paper would seem to be nearly nil. Ascocarps were never produced when cellulose was added to the agar substrate instead of placing bits of filter paper on the agar surface. The failure to obtain perfect stages of other fungus species may be due to the fact that isolates have been paired only on agar substrates.

Ullstrup's studies on the reaction of selected lines of corn to isolates of *H. carbonum* have indicated the presence of 2 races (9). The finding of the perfect stage of the pathogen will make possible a study of the genetic mechanism controlling pathogenicity. Successful crosses of race 1 × race 1, race 1 × race 2, and race 2 × race 2 have been obtained as part of the present studies, and ascospore progenies are being evaluated.

The study of evolution of sexuality in the fungi, particularly a genetic explanation for homothallism and heterothallism, has gained considerable attention in recent years. It is known that some genera of the fungi have both heterothallic and homothallic species. In *Cochliobolus*, some species are suspected of being homothallic. At least 1 species, *C. kusanoi* (Nisik.) Drechs. ex Dastur, was proved homothallic through culture studies of ascospore isolates. As the patterns of sexuality of a number of species within a genus are identified, a broader basis will be provided for studying evolutionary trends by means of interspecific crosses.

North Carolina State College
Raleigh, North Carolina

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A METHOD OF FREEING WHITE CLOVER PLANTS OF BEAN YELLOW MOSAIC VIRUS [1]

L. W. Baxter, Jr., and N. E. McGlohon [2]

SUMMARY

A method was devised to free certain clones of white clover from the bean yellow mosaic virus. Terminal cuttings 1 cm long or less were obtained from clones grown at 10°C. The cuttings were rooted in sterilized sand in glass jars with the mouth covered with polyethylene. About 6 weeks at 10°C in diffuse fluorescent light were necessary for rooting, after which cuttings were grown 6 weeks in a greenhouse at 25°C. Cuttings were then indexed on bean plants (var. Bountiful). Of 362 cuttings from 45 clones grown at 10°C., 126 were virus-free. In contrast, only 18 of 317 field-grown cuttings of the same clones were virus-free.

Breeding clones of white clover (*Trifolium repens* L.) frequently become infected with various aphid-borne viruses, particularly the bean yellow mosaic virus (BYMV) and alfalfa mosaic virus. Kreitlow et al (5) established that these viruses may seriously reduce the production of white clover.

In crop improvement work it is often desirable to compare the progeny of a single plant with the parents. Since most viruses of this nature are not seed-borne, and, as established by Kreitlow and Hunt (4), there is no evidence suggesting that these 2 viruses are seed-borne in this plant, it is important to keep the parent plant virus-free for accurate comparisons between parent and progeny.

Many breeding clones of white clover become infected by either or both viruses during growth and subsequent vegetative propagation. At Clemson many of the more desirable clones are currently infected with BYMV. This study presents results obtained from attempts to eradicate BYMV from some infected clones.

Materials and methods.—Forty-five clones of white clover infected with BYMV were chosen for this study.

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[2] Associate Plant Pathologist, South Carolina Agricultural Experiment Station, and collaborator ARS, U. S. D. A.; and Research Assistant, South Carolina Agricultural Experiment Station, respectively.

The plants were grown in 8-in. clay pots in a greenhouse until they were well established and had completely covered the soil surface in a pot. All plants were then cut back with scissors sterilized in 70% ethanol, so that no foliage was present, although stolons were left intact. The pots were then transferred to a controlled-temperature chamber at 10°C equipped with fluorescent lights adequate for growth of the clover. Water was applied to the plants at 10°C to avoid raising the temperature of soil or stolons.

After about 2 months, growth was adequate for cuttings. Ten terminal cuttings, each about 10–15 mm long exclusive of leaf and petiole, were taken from each plant and placed in wide-mouth, 1-gal. glass jars containing moist sterilized sand. The sand was firmed about each cutting by adding water. Each jar was then covered with a sheet of thin, transparent polyethylene held in place by a rubber band. The cuttings were held at 10°C in diffuse fluorescent light for about 6 weeks, when they were adequately rooted for transfer to 4-in. clay pots containing methyl-bromide-treated soil. The potted plants were placed in a greenhouse at about 25°C.

After the cuttings had grown for about 6 weeks in a greenhouse, each clover plant was indexed separately on 5 bean (*Phaseolus vulgaris* L., var. Bountiful) seedlings by the expressed-juice-carborundum technique. The bean seedlings were inoculated prior to the development of the first trifoliate leaf. Clover plants suspected as being virus free after the first test were reindexed at 30- and 60-day intervals.

For comparison with the low-temperature method, ten cuttings were made in June, 1957, from each of the 45 virus-infected clones grown under field conditions. Except for the original growth period, all cuttings were handled similarly.

RESULTS.—Of 450 tip cuttings made from plants grown at low temperature, 362 (80.4%) survived. Of a comparable number of cuttings made from field-grown plants, 314 (69.1%) survived.

The low-temperature method was more effective than the field method in eliminating BYMV from white clover. In the low-temperature treatment 55% of the clones and 34.8% of the cuttings were freed of virus, whereas only 8.9% of the clones and 5.7% of the cuttings from field-grown plants indexed virus free. A high percentage of cuttings of 4 clones grown at low temperature were freed of BYMV. Some clones were not freed of virus by this technique.

DISCUSSION AND CONCLUSIONS.—These data demonstrate that some virus-infected clover clones may be freed of BYMV by selecting tip cuttings. Holmes (1) eliminated the spotted wilt virus from dahlias by propagating tip cuttings. In a later paper he (2) described elimination of the aspermy virus from the Nightingale chrysanthemum. In that work he used tip cuttings as scions grafted onto uninfected chrysanthemum stocks. Of 244 scions tested, only 13 (5.3%) were freed of aspermy virus, and these still retained a second virus.

In the present investigation 18 (5.7%) virus-free cuttings were obtained from 317 field-grown plants. This figure approaches that obtained by Holmes with the aspermy virus. However, when the virus-infected plants were subjected to a controlled temperature of 10°C, 126 plants of 362 (34.8%) tested were virus free. Also pertinent here is that 25 clones were freed of BYMV by the low-temperature method in comparison to the 4 clones freed by the field tip-cutting method.

Despite repeated attempts, some clones were not freed of BYMV by either method. It is possible that the physiology of various plants differs so that some factor responsible for virus multiplication is liberated from the young developing tissue or is translocated from the older tissue. The latter hardly seems likely, however, since long stolon cuttings were made of some of the clones grown under cold-room conditions and some of these were found to be free of BYMV. In the case of such clones as one where only one plant of 7 was freed, it is possible that variation in the length of the stolon cutting could account for the difference. Similarly, if adequate numbers of tip cuttings were made of the 20 clones that were not freed of virus, it is possible that a low percentage might yield virus-free plants. It appears that clones differ greatly in the ease with which they may be freed of BYMV.

One other point that should be considered concerns 2 clones that yielded a high percentage of virus-free cuttings from field-grown plants but relatively few from the plants grown in the cold room. Kassanis (3) freed plants of certain viruses by growing plants at a continuous high temperature. Since the summer temperature in South Carolina is relatively high, it is possible that the physiology of these clones favors virus multiplication at a temperature lower than that provided by other clones.

Regardless of the mechanism responsible for virus multiplication, this study provides a technique whereby other infected plants may be freed of viruses.

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COLLETOTRICHUM BLIGHT OF DWARF MISTLETOE [1]

J. R. Parmeter, Jr.,[2] J. R. Hood,[3] and R. F. Scharpf[3]

SUMMARY

An undescribed disease of dwarf mistletoe, *Arceuthobium campylopodum* f. *abietinum*, was found in California. The disease, caused by *Colletotrichum gloeosporioides*, rapidly killed mistletoe shoots in all stages of development. The endophytic system was also invaded. In areas where infection was intensive, fewer than 20% of the mistletoe cankers bore healthy clumps of shoots. The causal fungus grew well on agar medium but sporulated abundantly only in liquid medium. Spores germinated over a wide temperature range. These characteristics suggested that the fungus might have some possibilities as a biological control agent.

INTRODUCTION.—In the spring of 1958, a severe blight of the shoots of *Arceuthobium campylopodum* Engelm. f. *abietinum* (Engelm.) Gill on red fir (*Abies magnifica* Murr.) was noted in the northern Sierra Nevada mountains of California. The symptoms were unlike those of any described disease of dwarf mistletoes. The fungus involved was not one of the known pathogens on this host. Since dwarf mistletoes are highly destructive on western conifers, the possibility that this fungus might effect some biological control made further study of the disease desirable. The initial results of that study are presented here.

RESULTS.—*Symptoms.*—Symptoms first appeared as dark-brown to black lesions, usually at the nodes of the shoot. These lesions enlarged until the entire shoot was affected (Fig. 1). The surface of the lesions was roughened by numerous acervuli surrounded by ragged, yellow fringes of the ruptured epidermis or "Cuticularepithel," as the outer, secondary cell layer of the mature shoot has been termed by Damm (*in* 4). Shoots in all stages of development were killed. These dead shoots became shriveled, and the lesions often turned gray. Many of the dead shoots remained attached to the cankers through at least one growing season.

Causal organism.—The causal fungus agreed with the description of *Colletotrichum gloeosporioides* Penz., *sensu* von Arx (1). Acervuli (Fig. 2) were distributed densely over lesions on all parts of the mistletoe shoot, including stems, leaves, buds, and fruit. No setae were found associated with acervuli on infected shoots, but when thin slices of fir bark from cankers bearing infected shoots were placed on water agar in Petri dishes, superficial acervuli with setae developed in the area of the cortical strands of the mistletoe endophytic system. Under moist conditions, salmon to cream-colored masses of spores exuded from acervuli on infected shoots. These spores were hyaline, 4.5–5.5 × 15.0–18.0 μ, and single-celled, becoming 2-celled at the time of germination. On water agar, germination exceeded 95% after 24 hours at 16°, 22°, or 30°C, with maximum germ tube length at 22°C. After 48 hours, germination was slight at 10°, and no germination had occurred at 4°C. Dark appressoria characteristic of *Colletotrichum* species were produced on germ tubes of spores germinated on the host or on Parlodion (cellulose nitrate) membranes (Fig. 3).

The fungus grew well on potato-dextrose agar (PDA), forming white to mottled gray colonies. Little or no sporulation occurred on PDA, 1/10 or ½ strength PDA, or on propylene-oxide-sterilized pea straw or mistletoe shoots on water agar held at room temperature. Incubation of colonies on PDA at 13°, 16°, 22°, or 28°C failed to induce sporulation. Further study disclosed a method by which the fungus could be induced to sporulate in culture.

Potato-dextrose liquid medium (15 ml in each

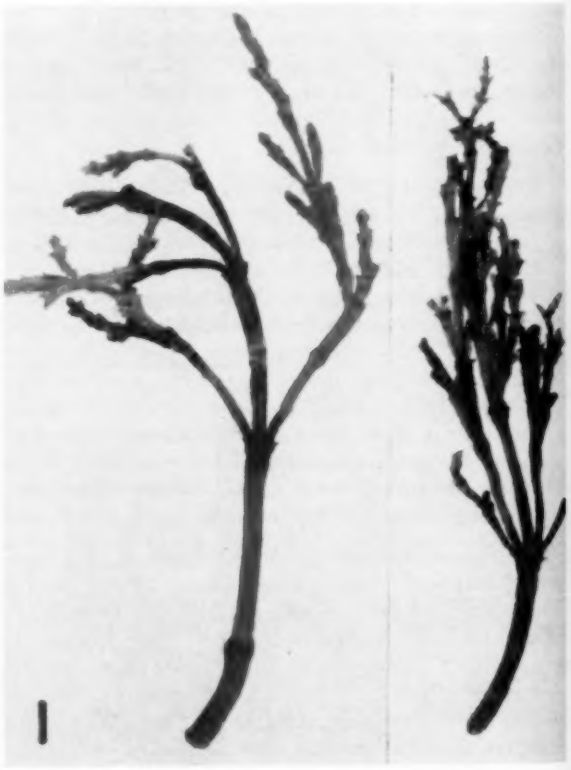

Fig. 1. Diseased shoots of dwarf mistletoe showing dark lesions caused by *Colletotrichum gloeosporioides*.

[1] Accepted for publication May 28, 1959.

[2] Assistant Professor of Plant Pathology, University of California, Berkeley.

[3] Research Assistant in Plant Pathology, University of California, Berkeley.

125-ml Erlenmeyer flask) was inoculated with mycelium from a 2-month-old colony grown on PDA. After 10 days' incubation at room temperature, the colonies in liquid began to sporulate abundantly. Successive crops of spores were harvested at 2–3 day intervals by washing the colonies with sterile water and collecting the resultant spore suspensions. This simple method of cultivation proved sufficient for the production of large quantities of inoculum.

Inoculation experiments.—On July 16, 1958, inoculations were made in the field on dwarf mistletoe shoots on red fir in an area where the disease was not present. Twenty cankers were tagged, of which 10 were covered with polyethylene bags. A spore suspension, prepared from naturally infected shoots incubated in a moist chamber, was sprayed onto the shoots of 5 of the bagged cankers and 5 of the exposed cankers. The remaining 5 bagged and 5 exposed cankers were sprayed with sterile distilled water. After 48 hours, the polyethylene bags were removed. By September 9, the inoculated shoots on all 5 bagged cankers and on 3 of the 5 exposed cankers had become diseased. Shoots on the control cankers remained healthy. Since these inoculations were made with spores from naturally infected shoots, additional inoculations with spores produced in culture were made to prove pathogenicity.

Inoculations were made in the greenhouse with spores produced by the culture method described earlier. Twenty-five branch cuttings of red fir and 20 of white fir (*Abies concolor* Lindl. & Gord.) bearing healthy mistletoe shoots were placed in vessels of water. On November 21, 1958, a dense spore suspension was applied with a sterilized camel's-hair brush to the shoots on 15 of the red fir cankers and 10 of the white fir cankers. The shoots on the remaining 10 red fir and 10 white fir cankers were painted with sterile distilled water. Symptoms began to appear on the inoculated shoots by December 4, and girdling lesions were apparent by December 12 (Fig. 4). The shoots on 13 of the 15 inoculated red fir cankers and on 7 of the 10 inoculated white fir cankers developed symptoms. All controls remained healthy. The fungus was reisolated from the diseased shoots.

Host-parasite relationships.—Sections of diseased shoots and of fir bark and wood beneath diseased shoots were cut with a sliding microtome, stained in acid fuchsin, dehydrated in a graduated series of ethyl alcohol solutions, cleared in xylol, and mounted in Permount. The fungus was found to invade all tissues of the mistletoe shoot. Mycelium was also observed in the endophytic system of the mistletoe. Since invasion of the endophytic system might be an important feature of biological control, studies were made to determine whether the fungus could be isolated from bark and wood containing the endophytic system.

Twenty-seven cankers bearing diseased shoots were prepared for isolation by cutting away the outer bark and all external mistletoe growth. Each canker was then swabbed with a 95% ethyl alcohol solution and flamed. At least 5 chips of bark and wood were then removed with a sterile knife and transferred to water agar in Petri dishes. These were examined daily, and any mycelium that appeared was transferred aseptically to test tube slants of PDA for identification. The mistletoe disease fungus was isolated from 12 of the 27 cankers. A second study was made, in which 5 cankers bearing diseased shoots were sawed in half. One half was sectioned for microscopic examination;

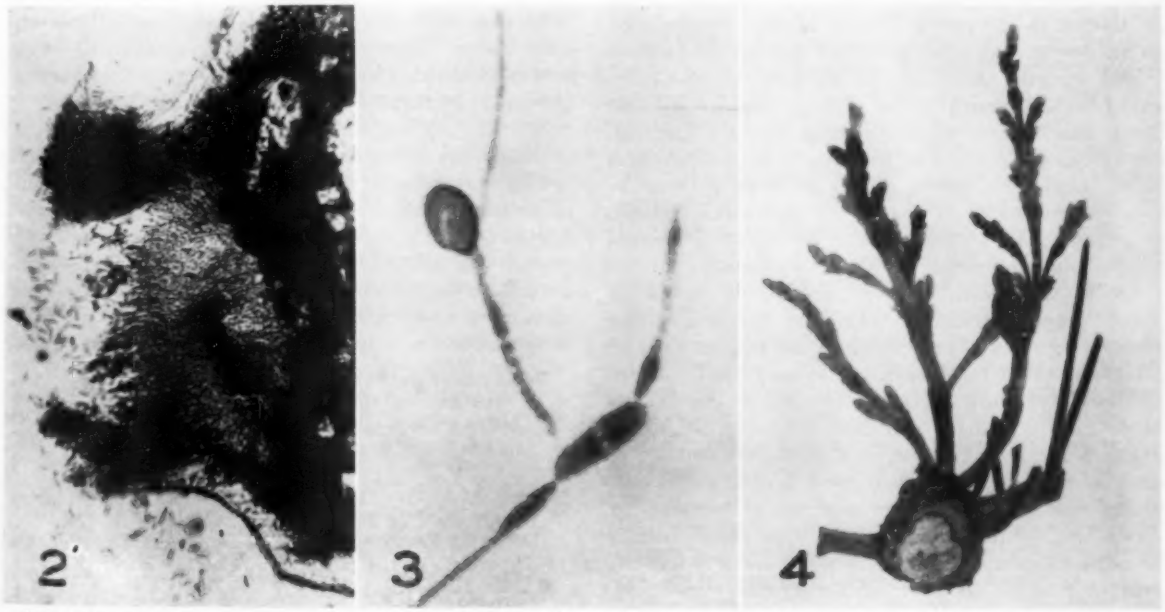

Fig. 2-4. 2) A cross section through an acervulus of *Colletotrichum gloeosporioides* on an infected mistletoe shoot. **3)** A germinating spore of *C. gloeosporioides* (lower right) showing the characteristic, dark appressorium (upper left). **4)** A mistletoe shoot, 3 weeks after inoculation, showing dark, developing lesions.

TABLE 1.—*The distribution of cankers with healthy clumps of shoots, diseased clumps of shoots, and no shoots with respect to the vertical distribution of cankers in the crowns of red firs*

Position with respect to vertical distribution of cankers[a]	Cankers bearing healthy clumps of shoots		Cankers bearing diseased clumps of shoots		Cankers with no shoots	
	No.	%	No.	%	No.	%
Top ¼	27	44.2	20	32.8	14	23.0
Upper-middle ¼	33	28.0	47	39.8	38	32.2
Lower-middle ¼	8	7.8	29	28.2	66	64.0
Bottom ¼	1	1.1	7	7.8	82	91.1
Total	69	18.5	103	27.7	200	53.7

[a] Position was determined by dividing into quarters the total vertical extent of mistletoe canker distribution in the crown in each of 14 trees. Figures are derived from the total number of cankers found in each quarter.

isolations were made from the other half. Again mycelium was found in the endophytic systems in the 5 cankers. The fungus was isolated from all 5 cankers. These observations indicated that the fungus had invaded the endophytic system.

Distribution.—The distribution and the intensity of infection on mistletoe shoots in individual red fir trees was investigated by examining and diagramming the location of all cankers on living branches of 14 trees, 15 to 35 feet tall. These trees were near but not under larger overstory trees in an area where the mistletoe blight was intensive. Of the 372 cankers examined, 18.5% had only healthy shoots, 27.7% had diseased shoots, and 53.7% had no shoots. The distribution of cankers bearing healthy or diseased shoots provided some insight into the nature of disease spread.

Table 1 shows the distribution of cankers with healthy clumps of shoots, diseased clumps of shoots, and with no shoots in relation to their position in the crown. These data were derived by dividing into quarters the total vertical extent of mistletoe canker distribution in the crown of each of the 14 trees. Percentages were based on the total number of cankers in each quarter. Although the absence of shoots appeared to be a result of the disease, cankers with no shoots were tallied separately from cankers that still bore diseased shoots. The percentage of cankers with healthy clumps of shoots decreased rapidly from the upper to the lower portion of the crown. Conversely, the percentage of cankers with no shoots increased rapidly from the upper to the lower portion of the crown. Cankers with diseased clumps of shoots occurred rather uniformly through the upper ¾ of the crown, with a lower percentage in the bottom ¼. Two factors appeared to account for these patterns of distribution: 1) The cankers in the upper portions of the crown are younger and have been exposed to the fungus for a shorter time, and 2) rain-disseminated fungi such as *Colletotrichum* species ordinarily cause greater damage in the lower portions of tree crowns.

Two areas of intensive infection were found, both in the northern Sierra Nevada. In these areas it was difficult to find shoots of mistletoe bearing seeds, apparently because few shoots survived for the 2 growing seasons necessary to produce seeds. No survey for additional areas of infection has been made.

DISCUSSION.—Present recommendations for the control of dwarf mistletoes in California (5), involving cutting or pruning of timber trees, are expensive and can be applied only to accessible, high-value timber stands. Throughout much of the western forest land, it is not physically or economically feasible to apply such control measures at present. A biological control of dwarf mistletoes might greatly reduce the damage from these pathogens over wide areas where present control methods cannot be applied.

Three fungus parasites of dwarf mistletoes have been described: *Wallrothiella arceuthobii* (Pk.) Sacc. (7), *Septogloeum gillii* Ellis (3), and *Metasphaeria wheeleri* Linder (6). Of these, little is known about *M. wheeleri*, and its possibilities as a biological control agent cannot yet be assessed. *W. arceuthobii* has been studied in some detail (8, 2), but successful cultivation and production of adequate inoculum for the establishment of epidemic infection have not been demonstrated. *S. gillii* sporulates readily in culture, and its possible use as a biological control agent has been suggested. The rate of disease development is, however, quite slow, about 7–8 weeks of incubation being necessary to produce visible lesions (3). *C. gloeosporioides* appears to be much more aggressive in this respect, requiring only 2–3 weeks to produce lesions involving the entire circumference of the shoot.

C. gloeosporioides possesses several other characteristics suggesting that it may have promise as a biological control agent. In culture it produces a sufficient number of spores for field application. The spores germinate over a wide range of temperatures and the disease seriously damages mistletoe shoots in areas where infection is intensive.

DEPARTMENT OF PLANT PATHOLOGY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

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SESAME BLIGHT CAUSED BY HELMINTHOSPORIUM SESAMI [1]

William J. Stone [2]

SUMMARY

A relatively long (60–72 hours) exposure to 100% relative humidity and a temperature of about 30°C resulted in good infection of sesame by *Helminthosporium sesami*. Age of the plants and of the pathogen in culture at the time of inoculation were found to be of great importance. Plants less than 21 days old were the most susceptible, and inoculum from cultures 7 days old caused the greatest damage to sesame. Soil fertility affected the severity of the disease. Only 14 of 267 sesame lines screened for resistance showed tolerance to *Helminthosporium* blight.

Miyake (3), in 1912, was the first to report *Helminthosporium* attacking sesame (*Sesamum indicum* L.). He published a description of the fungus and named it *Helminthosporium sesami* Miyake. In 1918 and 1919 Reinking (5, 6) reported *Helminthosporium sesameum* Sacc. on dead and dying stems of sesame in the Philippines and Southern China. It was not until 1940 that the pathogen was again associated with sesame. Mendez (2) found *Helminthosporium*, with *Alternaria*, attacking sesame plants in Costa Rica. He believed that *Helminthosporium* infected the plant after it was weakened by *Alternaria*. He described the *Helminthosporium* disease as first appearing in the young stem and then moving up the stem and attacking the leaf petioles and later the capsules. Watanabe (7), in 1941, reported that wounding the host was not requisite for infection. The organism was found to grow at temperatures from a maximum of 37° to less than 12°C. Optimum temperature was 27–30°, and optimum pH was 6.2. A *Helminthosporium* blight of sesame in the United States was first reported by Poole (4). During the summer of 1954, the fungus was found attacking plants in the field at College Station, Texas. Symptoms were prominent on stems, leaves, pedicels, and capsules. Poole described the stem lesions as ranging from small flecks 1 mm in diameter to large, sunken, dark-brown spots 10×40 mm in size. Lesions on the capsules varied from small, dark-brown spots at the base of the pedicel to large, brown lesions that enveloped the capsule and caused loss of some seed. Leaf lesions varied from small brown spots 1 mm in diameter to large, elongated lesions of about 2×20 mm. The organism isolated from these infections was identified as *Helminthosporium sesami* Miyake. The present report is based on studies of that organism.

MATERIALS AND METHODS.—Inoculum was prepared by a modification of the method of Andrus (1), using mycelial fragments obtained by briefly macerating the fungus and the oat-dextrose agar medium from a 9-cm Petri dish with 50 ml of water in a Waring Blendor. Oat-dextrose agar medium was used in all tests unless otherwise indicated.

At the time of inoculation the plants were usually at the 4-leaf stage, attained in 15–20 days of growth. Seven or 8 plants were grown in each 1-gal. can of Norfolk sand. Fertilizer was applied at the equivalent rate of 40 lb./A. each of nitrogen (N), phosphoric acid (P_2O_5), potash (K_2O), and calcium (Ca) (40-40-40-40).

Plants were inoculated by spraying the fungal suspension with a compressed-air paint sprayer at a pressure of about 40 lb./in.^{2} The spray nozzle used produced a coarse, cone-shaped spray pattern.

The inoculated plants were placed in a chamber at 100% RH (relative humidity) for 60 hours. Notes on infection were taken about 10 days later.

Guacara, a Venezuelan introduction, was selected as the test variety because it was known to be susceptible.

EXPERIMENTAL RESULTS.—*Preliminary investigations.*—The growth of the fungus was compared on several media to determine the best medium for culturing. The media compared were oat-dextrose, potato-dextrose, nutrient, corn-meal, Lima-bean, and bean-pod agar media. Culture growth was greatest on the oat-dextrose medium, followed in sequence by corn-meal, Lima-bean, bean-pod, potato-dextrose, and nutrient agar.

The results of a test to determine the effects of temperature on mycelial development in culture agreed very closely with those reported by Watanabe.

Duration of exposure to 100% RH for host infection.—Intervals of 12, 24, 48, 60, and 72 hours were used to determine the period at 100% RH necessary for

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[2] Formerly Research Assistant, Department of Agronomy, A. & M College of Texas; now Research Pathologist, Oilseed and Industrial Crops Research Branch, ARS, U. S. D. A., and the Delta Branch of the Mississippi Agricultural Experiment Station, Stoneville.

TABLE 1.—*Effects of humidity, age of plants, and age of cultures on infection of sesame by Helminthosporium sesami*

Test[a]	% in 4 infection grades[b]			
	0	1	2	3
A) Hours at 100% RH				
12	100			
24	98	2		
48	20	30	32	18
60		22	22	56
72		7	24	69
B) Age (days) of plants at inoculation				
7				100
14				100
21				100
28			8	92
35			8	92
42			4	96
49			33	67
C) Age (days) of cultures at inoculation				
7			11	89
14		88	8	4
21	89	11		
28	75	25		

[a] Tests A and B = % of 24 plants; Test C = % of 32 plants inoculated.

[b] Infection grades: 0 = no infection. 1 = small elongated or pinhead spots. 2 = plant stunted, stems brownish and watery; large coalescing leaf lesions. 3 = plants killed.

maximum host infection. Twenty-four plants were inoculated for each time interval; 8 plants for each time interval were sprayed with sterile oat medium and used as controls. The inoculated plants were immediately placed in a humidity chamber at 100% RH. Twenty-four inoculated plants and 8 control plants were removed at the end of each time interval.

Infection was most severe when inoculated plants were 72 hours at 100% RH; however, severe damage was also produced after 60 hours of exposure (Table 1-A). Although leaves of inoculated plants exposed 48 hours to 100% RH developed infection comparable with that of plants exposed for 60 and 72 hour periods, stems of these plants were only slightly damaged. The only symptom evident on plants exposed 24 hours was a very slight leaf infection. Plants that remained in the humidity chamber for 12 hours were not infected.

Influence of age of the host plants on infection.—Sesame was planted at 7-day intervals for 49 days to determine the effect of plant age on degree of infection. Seven days after the seedlings of the last planting had emerged, 24 plants of each age group were inoculated with a mycelial suspension of the fungus, and 8 were sprayed with sterile medium as controls.

Ten days after inoculation, over 90% of the plants that were 28, 35, and 42 days old were killed by the fungus (Table 1-B). Only 67% of the 49-day-old plants were killed. The older plants were apparently more tolerant than the younger ones. All the plants inoculated when 7, 14, and 21 days old were killed. Control plants were not affected.

Influence of age of the culture on degree of infection.—Five Petri dishes with 20 ml of medium in each were inoculated at 7-day intervals for 28 days to determine the influence of age of culture on degree of infection. Thirty-two plants were inoculated with each culture (7, 14, 21, and 28 days old). Controls (sprayed with sterile oat medium) were placed in the humidity chamber with each group of inoculated plants.

Pathogenicity of the culture decreased with age (Table 1-C). Cultures 7 days old killed 89% of the inoculated plants, and stem infection was severe on the surviving plants. Plants inoculated with cultures 14 and 21 days old showed only slight infection, but with the former causing more damage than the latter. Plants were only slightly infected by the cultures that were 28 days old.

Influence of plant nutrients on infection.—All possible combinations of nitrogen (N), phosphoric acid (P_2O_5), potash (K_2O), and calcium (Ca) were applied to an infertile Norfolk sand at the equivalent rate of 40 lb./A. of each nutrient to study the effects of soil fertility on infection. Sixteen plants for each combination were used.

Nitrogen showed a high increase in infection, and calcium showed a tendency to increase infection (Table 2). Plants showing the least infection were those grown on soil fertilized with K_2O alone, with P_2O_5 alone, and with the combination of P_2O_5 and Ca. Plants with the greatest amount of infection were those fertilized with N alone, and those with N and Ca in combination. Plants that received no fertilizer were also extensively infected.

Screening for host resistance.—One or more selections were chosen from each family of lines in the Texas sesame breeding program for determining whether any lines were resistant to *H. sesami*. Plants of all lines were inoculated 3 different times, using 8

TABLE 2.—*Effects of nitrogen, phosphorous, potassium, and calcium on infection of sesame by Helminthosporium sesami*

Fertilizer[a]				% [b] in 4 infection grades[c]			
N	P_2O_5	K_2O	Ca	0	1	2	3
0	0	0	0			13	88
0	0	0	40			31	69
0	0	40	0		50	29	21
0	0	40	40		7	21	72
0	40	0	0		40	26	34
0	40	0	40			64	36
0	40	40	0			21	79
0	40	40	40		11	11	78
40	0	0	0				100
40	0	0	40			7	93
40	0	40	0		13	19	69
40	0	40	40		6	6	88
40	40	0	0		19	7	64
40	40	0	40		6	13	81
40	40	40	0		6	19	75
40	40	40	40			23	77

[a] With or without the equivalent of 40 pounds of each nutrient per acre.

[b] Per cent of 16 plants inoculated.

[c] Infection grades: 0 = no infection. 1 = small elongated or pinhead spots. 2 = plant stunted; stems brownish and watery; large coalescing leaf lesions. 3 = plants killed.

TABLE 3.—*Infection of sesame lines and new introductions having possible tolerance to Helminthosporium blight*

Pedigree	%[a] in 4 infection grades[b] 0	1	2	3
Y 7-B-1-B-1-1		20.8	13.8	65.4
V 51-6-B-1-1-1		22.7	11.1	66.2
PI 153417-1-1-B-1-B-2-1		17.5	8.0	74.5
V 54.89-1-1		7.5	17.9	74.6
T 53177-7-64-4-1		13.0	13.6	73.4
T 53178-2-10-1-1		37.5		62.5
T 54124-1-4-1		45.8	8.3	45.9
T 54124-6-3-1		16.6	4.1	79.3
T 54058-B-1		20.8		79.2
T 54110-B-1		29.1		70.9
New introductions				
Haenan 9		25.0		75.0
PI 224647		75.0		25.0
PI 226303		100		
PI 229669		12.5	12.5	75.0

[a] Per cent of 24 plants, except for new introductions, which were based on 8 plants.

[b] Infection grades: 0 = no infection. 1 = small elongated or pinhead spots. 2 = plant stunted; stems brownish and watery; large coalescing leaf lesions. 3 = plants killed.

plants of each of the 267 lines selected at each separate inoculation.

Most of the plants were severely infected under the greenhouse conditions that existed. Of the 267 lines screened, none proved resistant or immune to attack by the disease when conditions for the disease were optimum. However, 14 lines, 4 of which were new introductions, showed some degree of tolerance to the disease (Table 3).

Although the number of plants killed in these lines was high, all had some plants that were not severely infected. The tolerance appears to be high in the new introduction selections, but because of a shortage of seed, these lines were screened only once. Thus their tolerance as reported here may be questioned.

Examination of the pedigrees of the 14 lines failed to reveal any common ancestor that may have been the source of tolerance or resistance.

DEPARTMENT OF AGRONOMY
TEXAS AGRICULTURAL AND MECHANICAL COLLEGE
COLLEGE STATION, TEXAS

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HELMINTHOSPORIUM ROSTRATUM INCITING EAR ROT OF CORN AND LEAF-SPOT OF THIRTEEN GRASS HOSTS[1]

Marvin D. Whitehead and Oscar H. Calvert

SUMMARY

Cross-inoculation studies were made with *Helminthosporium rostratum* on *Zea mays* and *Dactylis glomerata*, *Phalaris arundinaceae*, *Agropyron smithii*, *Bromus inermis*, *Festuca elatior* var. *arundinacea*, and *Cynodon dactylon*. Symptom expression on corn, including ear rot, and on 13 grasses in 11 genera was determined. In addition to the above, these include: *B. catharticus*, *B. commutatus*, *Panicum texanum*, *Leptachloa filiformis*, *Setaria viridis*, *Agrostis palustris*, and *Hordeum pusillum*. Conidial measurements are extended in range. Resistant turf strains of bermuda were noted.

A number of reports have been made on the occurrence of *Helminthosporium rostratum* Drechs. as a minor disease of graminicolous hosts. Its pathogenicity, however, has received little attention. *H. rostratum* was described in 1923 by Drechsler (1), from a leaf spot of *Eragrostis major* Host in Delaware. It was reported from Georgia in 1941 as a leaf spot of *Paspalum boscianum* Flügge, by Lefebvre and Johnson (2). Young, Lefebvre, and Johnson (7) later found it inciting leaf spots of corn (*Zea mays* L.); honey sorghum and Spur feterita (*Sorghum vulgare* Pers.); common and sweet sudan grass (*S. vulgare* var. *sudanense* (Piper) Hitchc.); and pearl millet (*Pennisetum glaucum* (L.) R. Br.). They reported considerable variation in lesions, but that they were generally small, 1–2×2.5 mm, and commonly limited laterally by the leaf veins. These lesions might coalesce to form large, necrotic areas, sometimes extend-

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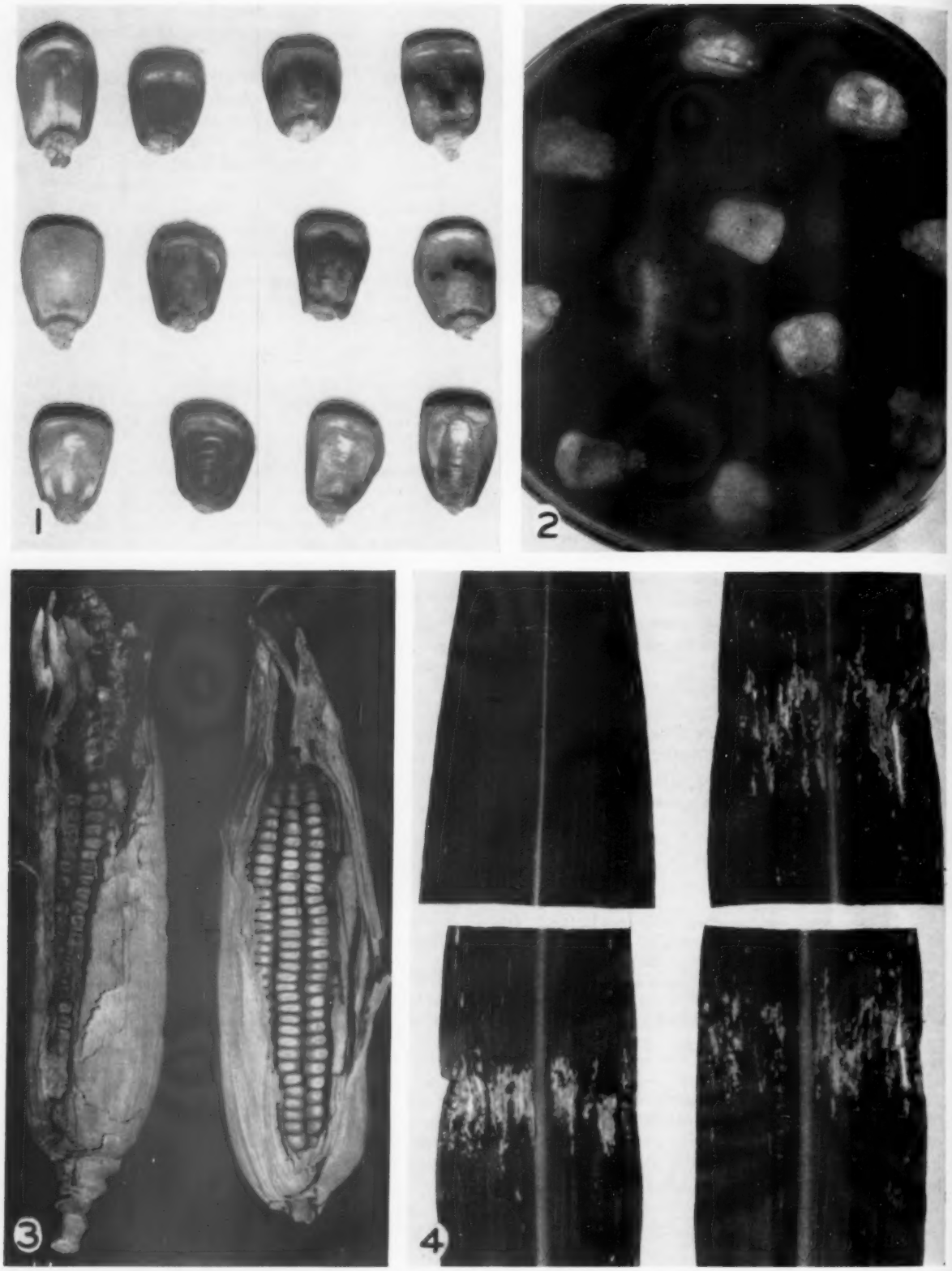

Fig. 1-4. Symptoms and cultural characters of *H. rostratum* on corn. 1) Healthy kernels compared with diseased, showing streaking to complete discoloration (×1). 2) Diseased kernels plated on PDA (×1). 3) Symptoms of ear and husk infection (×0.4). 4) Infected leaves compared with healthy (×0.6).

ing across the leaf veins. The necrotic centers of all the lesions, particularly corn, bleached to a straw color, usually with some browning at the edge and some yellowing at the ends. On pearl millet the lesions were at first dark brown and later tended to become lighter. Luttrell (3) found *H. rostratum* covering individual grains of pearl millet with a dense moldy mycelium. Nelson and Osborne (5) isolated *H. rostratum* from two samples of corn seed from North Carolina, one with 9% and one with 12% infestation. The organism was found fairly widespread in the soils of Georgia by Miller et al (4). In a soil fungi survey, 0.3% of the plates from forest soils and 2.8% of the plates from cultivated soils developed cultures of *H. rostratum*.

Drechsler (1) reported shorter, wider spores with fewer septations than those observed in this study of conidia from natural infection of 13 genera. He listed the morphological measurements of the conidia of *H. rostratum* as 14–22×32–184 μ, with 3–15 septations. In the present study the observed conidial measurements were 9.9–14.8×23.0–213.8 μ (mean 12.6×128.2 μ), with 3–23 (mean 11.9) septations.

Although most of the species of *Helminthosporium* have been reported to have a narrow host range, *H. rostratum* is herein reported to have a relatively wide host range.

Platings of numerous samples of corn seed from the corn breeding nurseries at College Station, Texas, revealed scattered infections by *H. rostratum*. Extensive platings of Texas sorghum seed in a sorghum storage study revealed 1–12% infection of *H. rostratum* in all samples plated. In 1954, seed from a field of certified Texas 17W (T585×61M)×(K55×K65) hybrid corn grown near San Antonio, Texas, when plated on potato-dextrose agar (PDA) showed 85% infection by *H. rostratum*, with almost no contaminating organisms (Fig. 2). Seed from this lot of corn had only 15% viability. Naturally infected ears appeared near normal, but shelled kernels were characteristically discolored, from the tip upward to the crown, by alternating bands of bright pink discolorations (Fig. 1).

A series of Missouri corn lines and the two single-cross parents of Texas 17W were inoculated in 1956 and 1957 at Huntsdale, Missouri, to determine the symptoms, range, and virulence of this pathogen on corn. Leaf inoculations were made by injecting 5 ml of spore-mycelial suspensions into the leaf whorls at weekly intervals from the time the plants were 2 ft. tall to the appearance of tassels. The inoculum was prepared by macerating *H. rostratum* cultures, sporulating on PDA in Petri plates, in a Waring Blendor with a small amount of water added. Tips of the ears in two series of experiments were inoculated by hypodermic injection of a spore-mycelial suspension, one at pollination time and one at the heavy dough maturity stage. Ears in another group were inoculated by placing sorghum grain cultures, prepared by the method described by Whitehead (6), at the tip and base of the ears, between the husk and ear.

Initial leaf infections from artificial inoculation of corn were at first water-soaked and dark green, and appeared sooner and initially appeared more pathogenic than infections incited naturally or artificially by the more common Helminthosporium leaf pathogens, *H. turcicum* Pass. and *H. maydis* Nishik. & Miy. After initiation of infection, however, the water-soaked portion of the lesions seemed to dry back, and from then on the receded lesions developed very slowly to a restricted reddish-tan rectangular lesion, 1–4×2 mm (Fig. 4).

The husks of severely infected ears from artificial inoculations were rotted and cemented to the kernels of the ears with the dark mycelium of *H. rostratum* (Fig. 3). Kernel discoloration ranged from a narrow light-red streak across the kernel near the crown to a light-red discoloration of all but the crown, and finally to complete charcoal-black rotting of the kernels (Fig. 1).

Since foliar infections on corn were restricted and the sporulation on these restricted lesions was scant, it was considered that the weed grass hosts in the corn fields of the area probably served as the major sources of inoculum for the ear infections. The three major

Fig. 5-7. 5) Leaf and culm symptoms on rescue grass (×0.7). **6)** Leaf lesioning of hairy chess (×0.7). **7)** Leaf lesioning of Colorado grass (×0.7).

weed hosts of the corn fields of Central Texas—rescue grass, *Bromus catharticus* Vahl.; Colorado grass, *Panicum texanum* Buckl.; and hairy chess, *Bromus commutatus* Schrad.—are very susceptible to the pathogen. Unlike the restricted sparse-sporulating lesions on corn, the symptoms on these common weed hosts are pronounced and showy. Heavy sporulation occurs on leaf lesions and dead infected tissues of these grasses.

The lesions on Colorado grass, 1–3×2–8 mm, soon develop tan centers and narrow purple margins (Fig. 7). There is little coalescing of lesions, but leaf blades become necrotic when infections are numerous.

The dark-brown spots on rescue grass, 1–4×3–12 mm, are surrounded by a yellow margin (Fig. 5). Lesioning similar in size and coloration occurs on hairy chess (Fig. 6); smooth brome, *Bromus inermis* Leyss.; tall fescue, *Festuca elatior* var. *arundinacea* (Schreb.) Schimm.; little barley, *Hordeum pusillum* Nutt.; and *Leptachloa filiformis* (Lam.) Beauv. The lesions on these species coalesce, inciting rapid leaf killing from the tips inward. An additional symptom of *H. rostratum* infection of rescue grass is a dark brown to black discoloration of the nodal and lower-leaf sheath tissues, and dark spotting of the culms (Fig. 5).

The disease symptoms on green foxtail, *Setaria viridis* (L.) Beauv., consist of restricted, diffuse, purplish-brown spots up to one millimeter in diameter.

There is rapid spread of the infection of *H. rostratum* in orchardgrass, *Dactylis glomerata* L.; western wheat grass, *Agropyron smithii* Rydb.; and Reed canarygrass, *Phalaris arundinaceae* L. The water-soaked lesions, which later turn tan, soon spread across the leaf blade, inciting killing of the leaf portion distal from the lesion.

Certain strains of the two turf grasses—bermuda, *Cynodon dactylon* (L.) Pers., and seaside bent, *Agrostis palustris* Huds.—were found to be very susceptible to the disease. *H. rostratum* was found to be one of the incitants of the "melting out" disease of seaside bent. There is a rapid invasion of leaf tissues, leaving the entire blade water-soaked and bluish-green. The infected tissues soon dry out. On bermuda grass, the lesions, 1–1.5×3 mm, are similar to the leaf spotting of the other grasses. The dark brown necrotic centers are surrounded by a diffuse yellow band. Strains resistant to very susceptible to infection were noted to occur in the bermuda strains observation nursery at College Station, Texas.

Ten single-spore cultures of *H. rostratum* obtained from orchardgrass, Reed canarygrass, western wheatgrass, smooth brome grass, tall fescue grass, and bermuda grass collected at College Station, Texas, and cultured on PDA media in Petri plates were used to inoculate each of the other grass species. The inoculum was prepared similarly to that used to inoculate corn. It was atomized on the plants, and the plants were incubated 24 hours at high humidity. The cultures obtained from each of the grasses readily cross-inoculated to each of the other grasses. The cultures obtained from bermuda grass produced a heavier, more severe infection on all the grass hosts than cultures obtained from the other hosts when inoculated back on the same host. The wheatgrass and orchardgrass cultures were next in order of virulence. In all cases the artificial inoculations produced symptoms on the plants similar to those observed in natural infections.

Department of Field Crops
Missouri Agricultural Experiment Station
Columbia, Missouri

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THE CHEMOTHERAPY OF WHEAT AND BEAN RUST DISEASES WITH SYDNONES[1]

D. Davis, H. J. Becker, and E. F. Rogers

SUMMARY

3-phenylsydnones inhibit the development of established infections of bean and wheat leaf rust. Chlorine substituents in the aromatic ring increase chemotherapeutic activity against wheat leaf rust while bean rust control is decreased by most substituents in the aromatic ring. Methyl, chloro, and bromo substituents at the 4 position inactivated 3-phenylsydnone in controlling wheat leaf rust but not bean rust. The 3-phenylsydnones show poor antifungal activity *in vitro*. Furthermore, they are structural analogs of phenylalanine and glycine. This suggests that they inhibit disease by altering host metabolism.

Chemotherapeutic agents for control of cereal rusts have been studied by a number of investigators. Gassner and Hassebrauk (2) and Hassebrauk (5, 6) found a wide variety of chemicals that inhibited cereal rust diseases when applied to the soil. Hart and Allison (4) substantiated the German researchers' observations on the effectiveness of picric acid and *p*-toluolsulfonamide and also demonstrated that sodium bromide controlled wheat stem rust when applied to the soil. Foliage application of chemotherapeutants was studied by Hotson (7). He demonstrated that a number of sulfa drugs, picric acid, and saccharin inhibited wheat stem rust. Again, Hassebrauk's (5, 6) observations were verified. In field trials, Livingston (8) demonstrated that foliage applications of calcium sulfamate and Acti-dione (3-[2-(3,5-dimethyl-2-oxycyclohexyl)-2-hydroxyethyl] glutarimide) markedly reduced wheat stem and leaf rusts. However, both compounds were moderately phytotoxic. More recently, Hacker and Vaughn (3) reported that foliage applications of the semicarbazone and oxime of Acti-dione effectively retarded wheat stem rust without seriously injuring the host.

This is the first report on the biological activity of meso-ionic compounds, specifically N_3-substituted sydnones.

METHODS AND MATERIALS.—The experiments described herein were designed to measure the rust eradicating properties of the sydnones and their precursors. Accordingly, the plants were treated 3 days after inoculation. By the fourth day, symptoms were already apparent on untreated plants. The test plants were *Triticum compactum* Host (Little Club wheat) and *Phaseolus vulgaris* L. (Pinto bean). When the second leaf of the wheat plant and the first trifoliate leaf of the bean plant were emerging, they were respectively inoculated with uredospores of *Puccinia rubigo-vera* (DC.) Wint. f. sp. *tritici* (Eriks.) Carleton, race 9, and *Uromyces phaseoli* (Pers.) Wint. var. *typica* Arth. The wheat plants were inoculated by first wetting the foliage with a 0.05% solution of Tween 20 and brushing the foliage with diseased leaves containing ripe uredinial pustules. The wheat plants were then placed at 100% relative humidity and 70°F for about 20 hours. The bean plants were similarly inoculated except that the upper surface only of the primary leaves was sprayed with a uredospore suspension containing 80,000 spores per ml. A local-lesion count was made 10 days after inoculation. There was an average of 80 pustules per leaf on the untreated inoculated wheat plant. Each compound was applied to plants in 3 pots containing 5 plants per pot. The pustules on the untreated inoculated bean leaves varied between 100 and 300 lesions per leaf. Two bean plants were used per treatment. The compounds that inhibited wheat leaf rust were tested at least twice. Likewise, the compounds that reduced bean rust and were not highly phytotoxic were tested at least twice. The active compounds were assayed in dosage series: 1000 ppm, 500 ppm, and 250 ppm. Solutions and suspensions of the test compound were prepared by dissolving 0.1 g in 2–3 ml of acetone, adding 0.05 g of Tween 20, and bringing to 100 ml volume with distilled water. Dilutions were made from the 1000-ppm stock suspensions. The suspensions were sprayed to run-off on both leaf surfaces with a No. 152 DeVilbiss atomizer at 20 lb. pressure.

RESULTS.—The data in Table 1 demonstrate some interesting relations between structure and activity of the sydnones. 1) All the sydnones investigated were substituted at the 3 position. Only the 3-phenyl substituents reduced bean and wheat leaf rust. 2) Of the substituents on the aromatic ring of 3-phenylsydnone, only chlorine enhanced chemotherapeutic activity against wheat rust. In the case of bean rust, all substituents except *o*-ethyl reduced chemotherapeutic activity. 3) Methyl, chloro, and bromo substituents at the 4 position inactivated 3-phenylsydnone in controlling wheat rust but not bean rust. However, it is interesting that sydnones with chloro or bromo substituents at the 4 position were not active against bean rust if the phenyl group was replaced with a *p*-chlorophenyl group at the 3 position. 4) Two intermediates in the synthesis of 3-phenylsydnone—*N*-phenylglycine and *N*-nitroso-*N*-phenylglycine—failed to inhibit either wheat or bean rust. These compounds are hypothetical hydrolysis products of 3-phenylsydnone.

Some of the more potent wheat and bean rust inhibitors were evaluated in the control of crown rust of oats (*Puccinia coronata* Cda. var. *avenae* Fraser & Ledington) and Early blight (*Alternaria solani* (Ell.

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TABLE 1.—*Chemotherapeutic activity of syndnones applied to wheat and bean foliage at 500 ppm 3 days after inoculation with uredospores*

Sydnone substituents		Disease index[a]		Phototoxicity on bean[b]
R	R'	wheat rust	bean rust	
phenyl	hydrogen	++	++++	Sl–M
o-chlorophenyl	hydrogen	++++	+++	S
m-chlorophenyl	hydrogen	+++	+++	M
p-chlorophenyl	hydrogen	+++	-	M–S
2,4-dichlorophenyl	hydrogen	+++	+++	M–S
3,4-dichlorophenyl	hydrogen	+	-	N
p-bromophenyl	hydrogen	-	+	N
p-nitrophenyl	hydrogen	-	-	N
p-methylphenyl	hydrogen	-	+	N
p-methoxyphenyl	hydrogen	-	-	N
p-sec-amylphenyl	hydrogen	-	-	N
o-ethylphenyl	hydrogen	-	++++	S
phenyl	methyl	-	+++	Sl
phenyl	nitro	-	-	N
phenyl	chloro	+	++++	S
phenyl	bromo	-	++++	Sl
p-chlorophenyl	chloro	-	-	N
p-bromophenyl	bromo	-	-	N
methyl	hydrogen	-	+	N
benzyl	hydrogen	-	-	N
cyclohexyl	hydrogen	-	+	N
β-naphthyl	hydrogen	-	+	N

[a] ++++ = 100–76% disease reduction (none or few 0;-type lesions); +++ = 100–76% disease reduction (many 0;-type lesions); ++ = 75–51% disease reduction; + = 50–10% disease reduction; - = 9–10% disease reduction.
[b] Sl = slight phytotoxicity; S = severe phytotoxicity; M = moderate phytotoxicity; N = no phytotoxicity.

& G. Martin) Sor.). Oat rust was inhibited by neither 3-phenylsydnone, 3-(*p*-chlorophenyl) sydnone, nor 3-(2,4-dichlorophenyl) sydnone. Early blight was not inhibited by 3-phenylsydnone, 3-(*p*-chlorophenyl) sydnone, 3-(3,4-dichlorophenyl) sydnone, or 4-bromo-3-phenylsydnone.

Characteristically, the chemotherapeutically active sydnones produced a slight formative effect on bean foliage. Chloro groups on the aromatic ring of 3-phenylsydnone increased the formative effect.

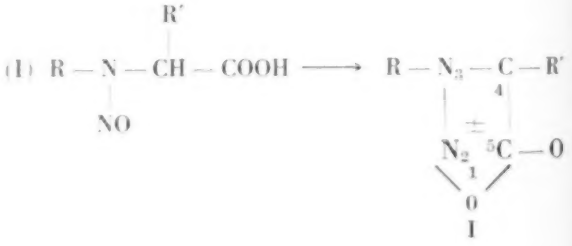
Translocation experiments indicated that the 3-phenylsydnones did not affect disease beyond the areas treated. Treatment of one primary bean leaf did not impart resistance to the adjoining primary leaf. Furthermore, treatment of both primary leaves did not affect disease in the first trifoliate leaf.

The first greenhouse experiments with the sydnones were performed in the winter. During this period no evidence of phytotoxicity was observed on bean. However, bean plants treated in May, June, and July were progressively more susceptible to leaf burn. Fortuitously, two of the most active bean rust inhibitors—3-phenylsydnone and 4-bromo-3-phenylsydnone—were the least phytotoxic. Thus, there was no direct correlation between the magnitude of rust inhibition and phytotoxicity. In contrast to bean, wheat was not adversely affected by the sydnones.

Field trials for the control of bean rust indicated that 3-phenylsydnone and 4-bromo-3-phenylsydnone applied at 1000 ppm at weekly intervals markedly reduced disease. However, considerable russeting was associated with the treatments. Extending the treatment intervals to 2 weeks and/or lowering the dosage resulted in poor inhibition of bean rust.

The sydnones were not fungistatic *in vitro*. Fungitoxicity data were obtained by a modified agar-plate paper-disk technique (9) using *A. solani*, *Fusarium oxysporum* Schlect. f. *lycopersici* Sacc., and *Pseudomonas solanacearum* E. F. Sm. as the test organisms.

DISCUSSION.—The meso-ionic sydnones are an unusual class of compounds in that they cannot be represented by neutral structures with normal valences; some unusual type of valence compensation exists. Formula I is a conventional reproduction of sydnones but is somewhat unsatisfactory because it suggests a zwitter-ion or betaine type structure. These relatively nonpolar heterocyclics are formed by cyclodehydration from N-substituted-N-nitrosoglycines; acetic anhydride and thionyl chloride-pyridine mixture are frequently used as cyclization agents.



Whether the sydnones inhibit disease as direct fungitoxicants or by some indirect mechanism has not been established. However, unless the sydnones are highly selective in their fungitoxicity, the negative correlation between *in vitro* and *in vivo* activity suggests that they increase resistance to disease by a mechanism that renders host tissues unfavorable to

disease development. First, the sydnones could be converted to a fungitoxic product in the host or induce the formation of a fungitoxic product from some native compound in the host tissues. Second, the sydnones might so alter the biochemical processes of the host that it could no longer support growth of the pathogen. The rusts, being obligate parasites with a high degree of physiological specialization, are dependent on the biochemical stability of the host. Since the 3-phenylsydnones are structural analogs of phenylalanine and glycine, they may act as competitive antagonists and thereby cause a metabolic imbalance in the host's tissues. Chemical disease control can be obtained with compounds that are nonfungitoxic but markedly affect host metabolism, as has been demonstrated in the Fusarium wilt disease of tomato. Nonfungitoxic plant growth regulators inhibited this vascular wilt disease only when treatment preceded inoculation (1).

The increase in toxicity to bean as the season progressed from winter to summer may be due to the decomposition of the sydnones to phytotoxic products. The sydnones rapidly turn brown on exposure to the sun. One possible breakdown product, NO_2, is very phytotoxic.

Merck Sharp & Dohme Research Laboratories
Merck & Co., Inc.
Rahway, New Jersey

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TRISTEZA VIRUS STRAINS IN RELATION TO DIFFERENT CITRUS SPECIES USED AS TEST PLANTS [1]

Theodore J. Grant

SUMMARY

Young Key lime, sour orange (selection #2), Eureka lemon, and Duncan grapefruit plants were similarly inoculated with comparable leaf tissue from standard sources of mild (T_1), strong (T_3), and severe (T_5) strains of tristeza virus. Differences in symptom expression were found to be related to the citrus species inoculated and the virus strain employed. Key lime was the best plant for detecting tristeza virus strains and strain mixtures. Leaf-piece transfers from all plants of the citrus species experimentally infected with the mild strain reproduced mild symptoms on the Key lime subculture plants, demonstrating the stability of this strain. Transfers from some plants of the different citrus species inoculated with the strong and severe strains showed a range in degree of symptoms on the Key lime subculture plants, indicating either alteration of the inoculum strains or the occurrence of mixtures of strains in the inoculum with separation or change in the virus strain ratios as a result of passage through the various citrus species.

Severe and mild strains of tristeza virus apparently occur in various citrus areas of the world (7, 10, 11, 13). West Indian limes (*Citrus aurantifolia* (Christm.) Swing.), also known as Key, Galego, or Mexican limes, have been used as common test plants for the detection of tristeza virus (15). Comparative inoculations with mild and severe strains of tristeza virus in Brazil (4) demonstrated differences in the symptoms produced on sweet orange (*C. sinensis* Osbeck) scions on sour orange (*C. aurantium* L.) rootstock and on Key lime plants. Detailed tests in Florida (9) and Texas (14) gave further evidence of the reproducible differential effects of tristeza virus strains on Key, or Mexican, lime plants.

In Australia (5) Eureka lemon (*C. limon* (L.) Burm. f.), Seville orange (*C. aurantium* L.), and grapefruit (*C. paradisi* Macf.) were used as test plants for detecting virus infection in field trees where tristeza disease occurred. Yellowing of these inoculated test plants was ascribed to a virus (seedling yellows) distinct from tristeza. In nature, however, seedling yellows virus was not found unaccompanied by tristeza

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TABLE 1.—*Response of 4 species of citrus to 3 strains of tristeza virus (standard inoculum), and of Key lime plants to subinoculation from each of the 4 species*

	Citrus species 10 mo. after inoculation								Key lime subcultures 6 mo. after inoculation							
	Plant growth (cm)[a]				No. stem pits per 10 cm[b]				Plant growth (cm)				No. stem pits per 10 cm			
Plant name	Control	T_1 mild	T_1 strong	T_3 severe	Control	T_1 mild	T_1 strong	T_3 severe	Control	T_1 mild	T_1 strong	T_3 severe	Control	T_1 mild	T_1 strong	T_3 severe
Key lime	347	297	143	39	0	1.6	156	400	264	206	87	19	0	3.2	176	400
Sour orange	171	198	146	52	0	0	19	0.08	264	214	114	51	0	2.4	112	281
Eureka lemon	207	201	193	21	0	0	0	0	264	219	227	61	0	2	2	190
Grapefruit	163	117	106	80	0	1.6	0.4	73	264	220	194	84	0	0.8	20	330

[a] Plants cut to single 6-in. main stem at time of inoculation; at end of test period the total length of all branches was recorded for each of the 5 plants in each treatment and averaged per treatment as an indicator of plant growth.

[b] At the end of test, bark was removed from main stem and main roots and most branches; the length of peeled stem and number of pits were recorded; and the average number of pits per 10 cm of stem was determined for each treatment. Where pits and striations were abundant, pit numbers were estimated.

virus. The conclusion that seedling yellows symptoms indicate a virus distinct from tristeza disagrees with the interpretation of results obtained in Brazil (2) and South Africa (12).

The tests described in this paper were undertaken to determine the reactions of four citrus species to infection with selected tristeza virus strains and strain mixtures in Florida.

MATERIALS AND METHODS.—The tristeza virus strains T_1 mild, T_1 strong, and T_3 severe have been employed previously, and their origin and characteristics described (9). Each strain was established in a Key lime plant that served as a standard source of inoculum.

Young, uniform seedling plants of Key lime, sour orange #2, Eureka lemon, and Duncan grapefruit were used as test plants. Twenty plants of each species were divided into 4 groups of 5 plants each. Group 1 served as uninoculated controls. Groups 2, 3, and 4 were respectively inoculated with the T_1 mild strain, the T_1 strong strain, and the T_3 severe strain. The inoculum was obtained by selecting 10 young, fully expanded leaves from the virus strain source plant. The leaves were randomized and numbered 1 to 10. Leaves 1 and 2 furnished 1 leaf piece each for inoculating plant 1 of each species, leaves 3 and 4 furnished 1 leaf piece each for inoculating plant 2 of each species, etc. This procedure was followed for each inoculum source and each test plant group to ensure uniform inoculum. All inoculations were made the same day by grafting the leaf pieces to the stems of the test plants. All plants were cut back to a single 6-in. main stem with only 3 leaves remaining at the upper part of the cut stem. Thus, all lateral branches represented new growth; the total length of these, compared with that of the uninoculated controls, indicated the growth response of the different citrus species infected with each of the tristeza virus strains.

Ten months after inoculation, the bark was removed from the main stem, main roots, and most of the branches. The total length of cleaned stem and number of pits were recorded, and the average number of pits per 10 cm of stem was determined for each treatment. When pits were confluent and formed striations, the numbers were estimated.

Four months after the inoculation of plants of the different species, subcultures were made in the following manner. A single leaf piece was taken from a fully expanded leaf in the upper portion of the leader branch of each plant. The leaf piece was grafted on a young Key lime plant cut back to a 6-in. single main stem bearing 3 top leaves. The subcultures were all made the same day. Records of growth and stem pitting on the limes were taken 6 months later.

RESULTS.—*Reaction of 4 citrus species after inoculation with 3 tristeza virus strains.*—Growth and stem-pitting data obtained from plants of the 4 citrus species are presented in Table 1. Stem pitting is illustrated in Fig. 1.

1) *Key lime.*—The T_1 mild strain reduced growth slightly and produced few stem pits. Vein-clearing symptoms appeared on a few leaves. The T_1 strong strain caused stunting, accompanied by abundant stem pits (Fig. 1-D), mass pitting, and striations close to points of inoculation. New terminal leaves showed vein clearing, followed first by cupping and yellowing and later by a degree of regreening. On Key lime the T_3 severe strain caused severe stunting. Stem pits were massed and formed striations on the short branches. The bark thickened, but the woody xylem of the main stem developed little after inoculation. Rootlet deterioration was evident at the end of the test. These results, similar to those previously reported (9), show that the tristeza virus strains caused consistent differences in the degree of severity of symptom expression on Key lime plants.

2) *Sour orange.*—The T_1 mild strain caused no reduction in growth and no stem pitting. The T_1 strong strain caused slight reduction in growth; the leaves showed occasional vein clearing, and stem pits were distinct (Fig. 1-C). The T_3 severe strain caused marked stunting of the sour orange plant, and the terminal leaves showed initial yellowing with a tendency to regreen. The bark was thickened and the xylem growth was poor or none. Very few stem pits were formed, but rootlet deterioration was evident.

3) *Eureka lemon.*—The T_1 mild and T_1 strong strains caused no reduction in growth and no stem pits. The T_3 severe strain caused yellowing of the leaves on subsequent new branches; growth of the plants then ceased and some regreening of the leaves

occurred. The bark was thickened, xylem development stopped, rootlets deteriorated, and no stem pits formed.

4) *Duncan grapefruit.*—The T_1 mild and T_1 strong strains caused some reduction in growth, but very few stem pits formed. The T_3 severe strain caused notable reduction in growth and very marked stem pitting (Fig. 1-B). Deficiency symptoms appeared on the leaves.

5) *Comparisons.*—The reactions by species show that the T_1 mild strain caused sufficient symptoms of vein clearing of leaves and pitting of the stems of Key lime for the detection of its presence, but this strain failed to produce distinctive symptoms on sour orange, Eureka lemon, or grapefruit even though its presence in these species was demonstrated by subcultures.

The T_1 strong strain caused sufficient symptoms on Key lime and sour orange that its presence was readily determined. It caused some reduction in the growth of grapefruit, and no symptoms on Eureka lemon.

The T_3 severe strain caused leaf yellowing, a marked reduction in plant growth, and an increase in rootlet deterioration on all the species tested.

Response of lime-plant subcultures of 3 virus strains from 4 different citrus species.—The T_1 mild strain was recovered from the inoculated plants of all species tested and remained unchanged by passage through these plants as measured by the degree of leaf and stem symptoms and growth. The T_1 strong strain was not materially altered by passage through Key lime and was only slightly altered by passage through sour orange, but it was notably altered by passage through Eureka lemon and grapefruit. Subcultures from the T_1 strong-stain-inoculated grapefruit and Eureka lemon caused little or no reduction in growth and few stem pits on the Key lime plants, indicating that a mild strain instead of the strong strain had been recovered. The T_3 severe strain was not altered by passage through Key lime plants but was altered by passage through Eureka lemon, sour orange, and grapefruit. While these results might indicate that the original inocula were single strains that had been altered, a more likely explanation is that strain mixtures were present in the T_1 strong and T_3 severe inoculum and that differences in virus strain ratios occurred as a result of passage through Eureka lemon and grapefruit hosts.

The observed responses of the inoculated Eureka lemon plants suggested that this species had a pronounced effect on the multiplication and distribution of the T_3 severe strain. Four Eureka lemon plants, infected for 36–64 weeks, were selected for study. The described method of subculturing on Key lime plants was employed. Eighteen leaf-piece inoculations from the 4 Eureka lemon plants produced severe tristeza on 7, strong tristeza on 2, mild tristeza on 4, and no symptoms on 5 of the Key lime subculture plants. In one instance, leaf pieces transferred from opposite laminae of the same Eureka lemon leaf resulted in one subculture with severe, and one subculture plant with mild, tristeza symptoms. When adjacent pieces of leaf tissue of another plant were used, one piece produced severe symptoms of tristeza and the adjacent piece gave no symptoms on the subculture plant. Of 4 Eureka lemon leaf pieces taken from young leaves, 2 caused severe tristeza and 2 no symptoms on the subculture plants. Of 14 leaf pieces from mature leaves, 5 produced severe tristeza, 2 strong, and 4 mild, and 3 produced no symptoms on the subculture plants.

These data show that, in the Eureka lemon plants tested, the T_3 tristeza virus was present in some but not in all leaves or all parts of the same leaf, even though the plants had been inoculated for 36–64 weeks. It is apparent that the Eureka lemon plants influenced multiplication and distribution of the T_3 severe tristeza virus. These results suggested that other citrus species, such as Key lime, with tissue reactions of vein clearing and stem pitting, might also influence virus multiplication and distribution, particularly if older plants with more branches and leaves were used.

To test this hypothesis, 5 Key lime plants (group A) were inoculated in the usual manner with one leaf piece each from 5 leaves taken from the standard T_3 source of inoculum. These 5 plants were cut back to single 6-in. main stems with 3 leaves remaining at the top of each. In contrast, another series of 5 larger Key lime plants (group B), with several branches 10–20

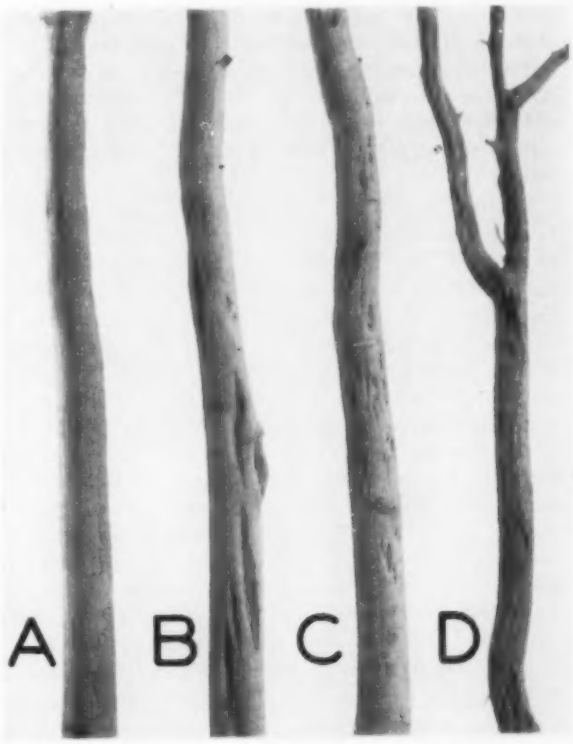

Fig. 1. Characteristic stem pitting symptoms on different citrus species 10 months from date of inoculation; **A)** Healthy (u inoculated) grapefruit, **B)** grapefruit inoculated with the T_3 severe strain, **C)** sour orange inoculated with the T_1 strong strain, and **D)** Key lime inoculated with the T_1 strong strain of tristeza virus. (Photograph by Dr. Gordon Grimm.)

in. long, were inoculated with pieces from the same T_3 inoculum source leaves, and were not cut back.

Ninety days after inoculation, subcultures were made in the usual manner from the plants in groups A and B with leaf pieces from young and old leaves, some showing vein clearing and some showing no symptoms. Tristeza symptoms on Key lime subculture plants were recorded 6 months after inoculation. The results of transfers from plants in group A were as follows: 5 leaf pieces from young leaves with vein-clearing symptoms produced severe tristeza; 5 leaf pieces from old leaves that showed no symptoms produced strong to severe tristeza on 2, mild tristeza on 1, and no symptoms on 2 subcultures. Group B results were as follows: 6 leaf pieces from young leaves with vein-clearing symptoms produced severe tristeza on the 6 subcultures; 4 leaf pieces from young leaves with no symptoms produced severe tristeza on 2, mild tristeza on 1, and no symptoms on 1 subculture plant; 5 leaf pieces from old leaves having a few scattered cleared veins produced severe tristeza on 3 and mild on 2 subculture plants; 9 leaf pieces from old leaves with no symptoms produced strong tristeza symptoms on only 1, mild tristeza on 3, and no symptoms on 5 subculture plants.

The data indicate that the T_3 severe strain moved readily into the young Key lime leaf tissue and was reproduced in subcultures from all young leaves showing distinct vein clearing from both the cut back (group A) and branched plants (group B). In contrast, the data indicate that virus movement into the old leaves was slower and that the mild or strong strain mixtures were more prevalent than the severe strain.

Discussion.—Wide differences in citrus species, variety, and hybrid reactions to tristeza virus have been recognized (8) and discussed with respect to virus multiplication and tissue reactions (2, 3). Likewise, the differential effects of severe and mild strains of tristeza have been noted (4, 14). The concept that tristeza virus can occur as strain mixtures in various proportions has also been reported (6, 9). A recent study of tobacco mosaic virus (TMV) by Cohen et al (1), using the U1 and U2 strains and electrophoretic methods, yielded knowledge of the movement and occurrence of different virus strain ratios of these TMV strains in *Nicotiana tabacum* L. leaves following inoculation with controlled mixtures of strains. Although the tristeza virus strains may not behave exactly like the TMV strains, the concept of different virus strain ratios and their variation within a single plant assists in obtaining a better understanding of the results obtained in the tristeza virus subculture tests.

In the present studies, for example, all the components of the severe strain may be judged to have maintained a balanced ratio and to have moved with food materials to the succulent new growth on the branched Key lime plants, but the same strain ratio was not maintained in movement into mature leaves. Virus movement into the mature leaves might be expected to be against the direction of food movement, and in these cases the mild strain predominated and appeared to be more invasive. The results obtained show, for example, that, in the Eureka lemon inoculated with the T_1 strong strain, subcultures from the terminal leaves produced symptoms typical of the mild strain. This and other indicated modifications in the T_3 severe strain by passage through grapefruit and Eureka lemon in comparison with passage through Key lime again emphasize the importance of different citrus species as media for the development and distribution of the different tristeza virus strains and strain mixtures.

On sour orange the strong strain caused stem pitting, the mild strain caused no pits, and the severe strain caused stunting and very few pits. Yet subcultures from these different strains from infected sour orange to Key limes caused a graded range in stem pitting slightly below, but similar to, that obtained with subcultures from the original inoculum sources on Key limes. These results demonstrate that stem pitting is not due to another virus but is only one of the symptoms of the tristeza virus related to host-tissue response of a given species and the tristeza virus or virus strain mixture present.

In 1949 it was reported (3) that young plants of sweet orange on sour orange rootstock showed symptoms sooner than older plants. Likewise, it was noted that tristeza symptoms were observed on young seedlings of 50 different citrus varieties following inoculation by means of aphids, and that it was easier to produce symptoms on young than on older seedlings. The results of present tests emphasize again the importance of differences in plant size and branched condition in relation to virus distribution. It should also be evident that sources of inoculum and methods of inoculation have important bearings on the results obtained.

Because of the several variables that may influence tristeza virus strain mixtures, the methods employed in the present studies of different citrus species and the results obtained have special significance. By using standard sources of inoculum and leaf pieces from paired leaves for the inoculation of young plants of the different citrus species and by similar treatment of the plants on the same date of inoculation, and by subsequently growing the plants on the same greenhouse bench, it was possible to reduce the influence of several variable factors and to compare with reasonable accuracy the plant species responses to the inoculum used. Thus, it seems logical to conclude that leaf yellowing and stem pitting were not due to viruses distinct from tristeza, but represented degrees of symptom expression of tristeza that varied in relation to the citrus species inoculated and the virus strain or strain mixtures employed.

Crops Research Division
Agricultural Research Service
U. S. Department of Agriculture
Orlando, Florida

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PHYTOPATHOLOGICAL NOTES

THE EFFECT OF VARIATIONS IN TEMPERATURE ON THE NECROSIS ASSOCIATED WITH INFECTION TYPE 2 UREDIA OF THE WHEAT STEM RUST FUNGUS [1]

William Silverman [2]

The reaction of Marquis wheat to race 38 of *Puccinia graminis* Pers. var. *tritici* Erikss. & E. Henn. is sensitive to temperature.[3] Marquis is moderately resistant at 65–75°F. and race 38 produces an infection type 2; the variety is moderately susceptible at 85–95°F. and race 38 produces infection type 3. Necrosis of wheat cells is characteristically associated with infection type 2, and a peripheral necrotic ring borders the mycelial area (the "green island") of each rust lesion. Since temperature appears to be a very important factor influencing necrosis of host cells, an experiment was made to determine the effect of temperature fluctuations during rust growth on the final development of the uredium and the necrotic ring.

Marquis seedlings were inoculated with race 38. After 24 hours in a moist chamber, the inoculated plants were divided into 2 groups and placed in controlled environment rooms: one group was at low temperatures (day temperature, 75°F; night temperature, 65°F) and the other group at high temperatures (day temperature, 95°F; night temperature, 85°F). On each day at the beginning of the light period, 3 representatives of each temperature group were reciprocally exchanged between the low and high temperatures. In this manner it was possible to obtain a series of plants each subjected to a change in temperature at some different stage during development of the rust uredium.

On control plants kept continuously at high temperatures (Fig. 1-A), the early fleck stage of rust developed 4 days after inoculation. Uredia were erumpent 7 days after inoculation, with primary sporulation abundant by the 8th day. Secondary sporulation developed on the 9th day. The necrotic ring was absent.

On control plants kept continuously at low temperatures (Fig. 1-B), the early fleck stage appeared 5 days after inoculation. Uredia were erumpent 8–9 days after inoculation, with primary sporulation well developed on the 9th day. There was no secondary sporulation. A peripheral ring appeared 7 days after inoculation and was judged necrotic by the 11th day.

If inoculated Marquis plants were transferred from high to low temperatures (Fig. 1-A, line 1) before any external symptoms of rust appeared, rust develop-

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[2] Formerly Research and Teaching Assistant, Department of Plant Pathology and Botany, University of Minnesota.

[3] Silverman, W. A toxin extracted from Marquis wheat infected by race 38 of stem rust (Puccinia graminis Pers. var. tritici Erikss. & Henn). Phytopathology (in manuscript).

ment was similar to that on plants continuously at low temperatures (Fig. 1-B, controls); the early fleck stage appeared 5 days after inoculation, primary sporulation was well developed on the 9th day, no secondary sporulation appeared, and a chlorotic ring appeared about 5 days after the transfer to low temperature.

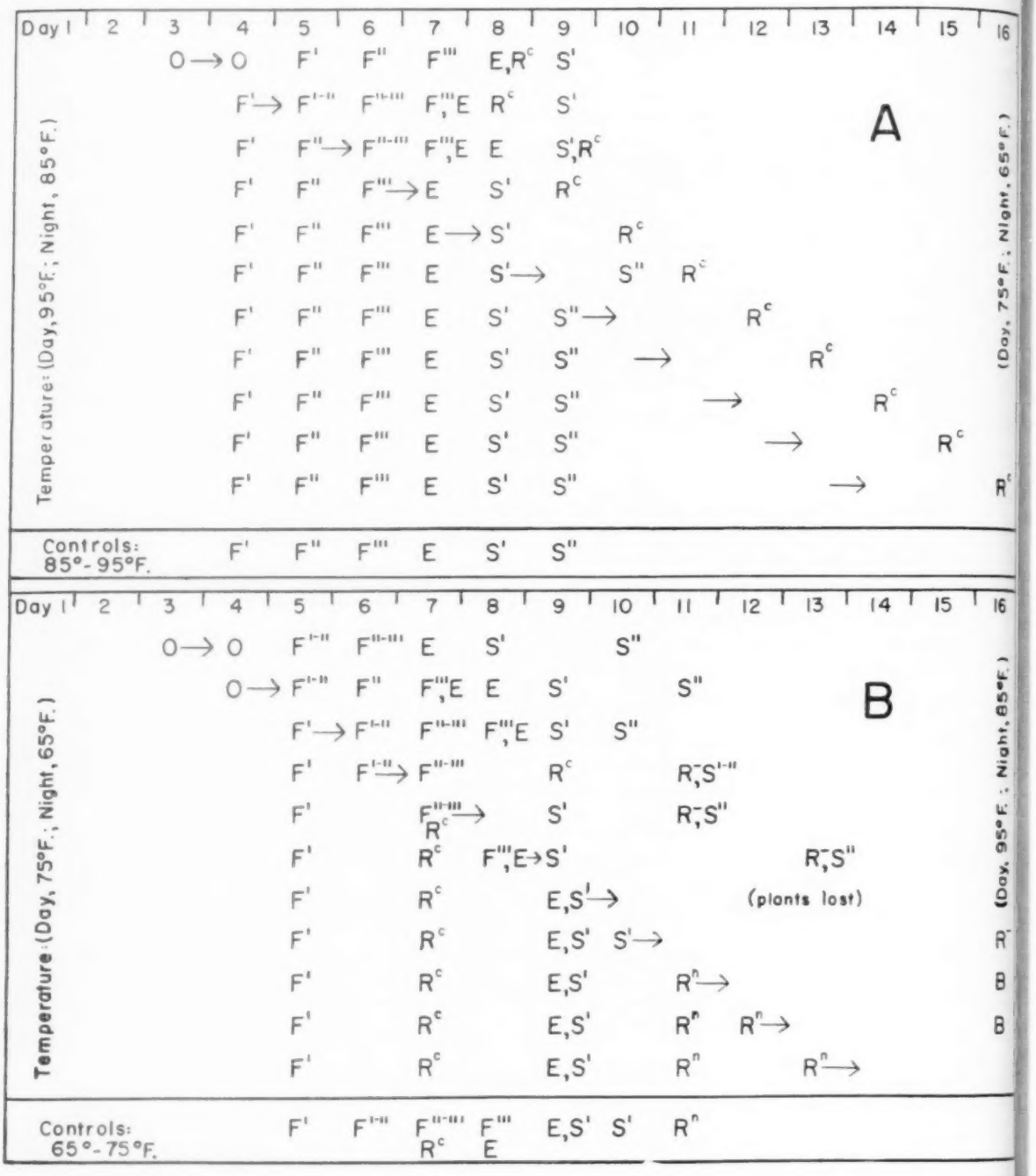
Day 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Temperature: (Day, 95°F.; Night, 85°F.)
(Day, 75°F.; Night, 65°F.)
A
Controls: 85°-95°F.
Day 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Temperature: (Day, 75°F.; Night, 65°F.)
(Day, 95°F.; Night, 85°F.)
B
(plants lost)
Controls: 65°-75°F.
O, No external sign of infection.
E, Rust uredia erumpent.
B, Lesion border dark green.
→, Plants transferred from one temperature to another.
F', F", F"' Flecking ---- early, mid, and late stages.
S', S" Sporulation abundant ---primary and secondary
R^c, R^n, R^- Ring chlorotic, necrotic, and absent.

Fig. 1. Development of *Puccinia graminis tritici* race 38 on Marquis wheat seedlings at different temperatures duri 16 days following inoculation.

When transfer was delayed until after rust sporulation had begun (Fig. 1-A, lines 6–11), the rust fungus sometimes produced secondary sporulation, and formation of the ring was accelerated so that it appeared 3 days after the transfer (Fig. 2-A).

The instability of the reaction of Marquis wheat to race 38 of stem rust was most evident in the plants transferred from a low to a high temperature. With very early transfer, before external rust symptoms appeared, the early fleck stage of rust appeared 5 days after inoculation, but, in addition, the mid-fleck stage also occurred on the same day (Fig. 1-B, lines 1–2). In other words, there was a telescoping of the early symptoms. The more precocious development was not apparent, however, if plants were transferred during the flecking stages of rust development (5–6 days after inoculation), nor did such transfers hasten erumpency and primary sporulation (Fig. 1-B).

Secondary sporulation was delayed by the initial low temperature, sometimes for as long as 7 days after plants were transferred to the higher temperature.

Variability in the peripheral chlorotic ring associated with the rust lesions was perhaps the most striking evidence of the instability of the reaction when inoculated plants were transferred from a low to a high temperature. The ring never appeared in plants transferred from low to high temperatures at any time during the first 5 days after inoculation (Fig. 1-B). In this respect the plants were comparable to those that had been at high temperatures continuously. If the transfer was on the 6th day, approximately at the mid-fleck stage of rust development, an evanescent pale-green chlorotic ring appeared 3 days after the transfer and persisted for only 2 days. If the chlorotic ring had formed before transfer of the plants on the 7th day after inoculation, the ring persisted 4 days; and if transfer was on the 10th day, the ring persisted 6 days. Apparently the physiologic processes within the cells of the leaf were reversible for a time, and the cells recovered from the chlorosis (Fig. 1-B, lines 4–6). By the 11th day, however, the processes were irreversible, the cells in the ring area had become truly necrotic, and in plants transferred at that time the ring persisted throughout the life of the leaf. In those plants a dark-green band soon appeared around the perimeter of the necrotic ring. The rust mycelium in the "green island" of the lesion had grown beyond the area of necrotic cells and had established itself beyond the outer edge of the ring, where it multiplied and again sporulated about 8 days after the transfer to high temperatures (Fig. 2-B).

Disease development is dependent on the interaction between host, pathogen, and environment. In the case of the rust-host association reported herein, the development of one aspect of the disease syndrome, necrosis of the host cells, is especially sensitive to a single environmental factor, the temperature prevalent during lesion development. In the early stages of infection, temperature may have merely quantitative effects on the vegetative growth of the rust fungus, as indicated by the extent of the mycelial area and the time required for the first disease symptoms to appear. Somewhat later, about when spore formation commences, qualitative differences attributable to the influence of temperature appear. Higher temperatures favor continued mycelial extension and abundant secondary sporulation; chlorosis or necrosis of host cells is absent at these elevated temperatures. Physiological processes that exert adverse effects on host

Fig. 2. Uredia of *Puccinia graminis tritici* race 38 on Marquis seedlings that were transferred from one temperature to another during rust development (×6). **A)** Plants transferred from high (85–95°F) to low (65–75°F) temperatures after extensive development of primary and secondary sporulation. The necrotic ring developed at the periphery of the mycelial area after 3 days at low temperature. Photographed 9 days after the transfer to the low temperature. **B)** Plants transferred from low (65–75°F) to high (85–95°F) temperatures after the necrotic ring developed. The sporulation outside the necrotic ring appeared after 8 days at the high temperature. Photographed 18 days after transfer to the high temperature.

cells and result in chlorosis and eventual necrosis, with consequent limitations of rust growth and sporulation, are favored by low temperatures; however, in the early stages of lesion development these adverse effects caused by relatively low temperatures may be counteracted by higher temperatures.—Department of Plant Pathology and Botany, Institute of Agriculture, University of Minnesota, St. Paul 1, Minnesota.

PYTHIUM SPECIES STILL LIVING IN MUCK SOIL AIR-DRIED SIX YEARS

Paul E. Hoppe

Information concerning the longevity of the *Pythium* species in dry soil was obtained from a pail of muck soil left to dry for 6 years in the laboratory under ordinary room conditions. The soil was the kind used by the writer in corn "cold" tests, in which 100% seed rot normally develops when untreated susceptible seed is tested. *Pythium* spp. are recognized[1] as the principal incitants of the disease in the soil.

The pathogenic potential of *Pythium* spp. in the dried soil was measured in this manner: untreated and captan-treated corn kernels, wounded at the crown to increase susceptibility to seed rot, were cold-tested in unreplicated 50-kernel lots in the soil after it was wetted to saturation with distilled water and then kept at room temperature for 0, 2, 5, 8, 10, 15, and 30 days before the tests were made. The rolled-towel technique[2] was used. The kernels, in contact with the soil, were incubated 8 days at 10°C. after which the "dolls" were moved to room temperature, where the germinations were recorded 3 days later. Recently collected muck soil was used as a control.

The data from the tests are given in Table 1. Seed treated with captan gave excellent germination in both soils, whereas the untreated kernels developed essentially 100% seed rot in the normal muck soil. In contrast, tests of the untreated seed in the 6-year dried muck resulted in little or no disease when the soil had been wetted for 0 or 2 days (Fig. 1). After 5 days in the wetted condition, germination of the untreated seed in the dried soil dropped to 81%, after 8 days to 77%, after 10 days to 14%, and after 15 or more days to essentially 0 (Fig. 1). Thus a wetting period of about 5 days was required for first evidence of activity of *Pythium* species, and 10–15 days of storage in a wet condition were required for the fungus to regain its full pathogenic potential.

The facts disclosed are of particular interest to those engaged in cold-testing seed corn. Warnings have been given against the use of dried soils, but no

[1] Hoppe, Paul E., and John T. Middleton. 1950. Pathogenicity and occurrence in Wisconsin soils of Pythium species which cause seedling disease in corn. (Abstr.) Phytopathology 40: 13.

[2] Hoppe, Paul E. 1955. Cold testing seed corn by the rolled towel method. Wisconsin Univ. Agr. Expt. Sta. Bull. 507, 5 p.

TABLE 1.—*Per cent cold-test germination of untreated and captan-treated corn kernels in normal muck soil, and in similar muck air-dried for 6 years and then kept wet for the days indicated prior to the test*

	Wet period (days)						
Kind of soil and seed	0	2	5	8	10	15	30
Recently collected unwetted muck:							
captan-treated seed	98	96	99	99	94	96	9
untreated seed	1	0	0	2	0	0	
Muck dried 6 years:							
captan-treated seed	97	94	98	96	98	97	9
untreated seed	97	96	81	77	14	1	

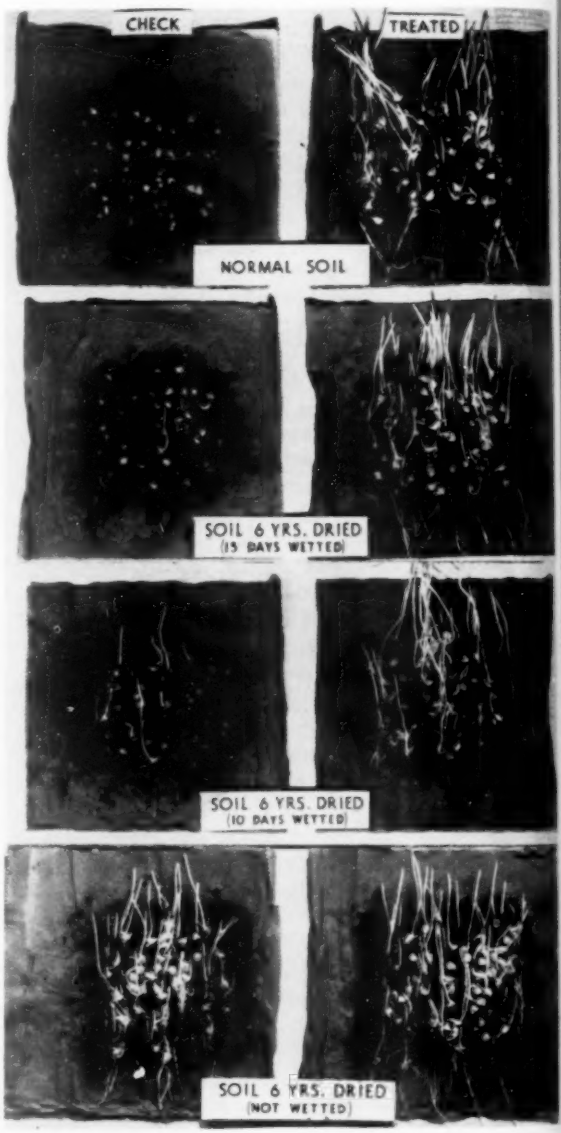

Fig. 1. Cold-test germination of untreated and captan-treated corn kernels in normal muck soil and in a similar muck air-dried 6 years after being wetted for the days indicated prior to the test.

actual data bearing directly on the problem have been published previously. These data substantiate the concept that thoroughly dried muck soil should not be used immediately for cold tests. However, they also indicate that such soil need not be discarded, but may be used after a 2-week wetting period.

Enough of the sample remains for future tests of the longevity of the *Pythium* species in the dessicated soil. —Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin.

HYPERPARASITISM OF A POWDERY MILDEW FUNGUS ON THE LONDON PLANE TREE

E. B. Himelick and Dan Neely

A powdery mildew fungus, identified as *Microsphaera alni* (DC.) Wint., was found on foliage of London plane trees, *Platanus acerifolia* Willd., for the first time in Illinois in 1958. Identification of the fungus was at first complicated by the constant presence of a fungus parasitic on the powdery mildew fungus. This parasitic fungus was identified as *Cicinnobolus cesatii* d By. This is believed to be the first report of *Microsphaera alni* on foliage of London plane in the Midwest and the first report of *M. alni* being parasitized by *C. cesatii* in the United States.

A large number of London planes and American sycamores growing on the University of Illinois campus and in Champaign-Urbana were examined in July and August of 1958. Observations indicated that London plane was more severely affected by powdery mildew than was American sycamore. It was difficult to find leaves of American sycamore affected with powdery mildew. Infection on London plane ranged from light to severe. Trees that showed severe infection had as much as 25–30% of the leaves affected with the powdery mildew fungus. Many affected leaves were stunted or malformed (Fig. 1). The heavy gray pubescence normally present on the lower surfaces of young sycamore leaves, which disappears sometime during the period of maturation, tended to remain on infected leaves. Conidial and cleistothecial structures of the powdery mildew fungus were rarely found during the summer and early fall.

A description of *C. cesatii* is given as it appeared on *Microsphaera alni*. In early stages of development, the small hyphae of *Cicinnobolus* are seen ramifying throughout the mycelium and condiophores of the powdery mildew fungus. Further development of the *Cicinnobolus* mycelium in the invaded conidiophores results in the formation of pycnidia. The wall of the host conidiophore swells to enclose a pyriform to globose pycnidium having a definite beak and ostiole. The pycnidia are reddish brown and measure 23–42 $\times$ 35–70 μ. Conidia are formed from short outgrowths of the cells lining the inner wall of the pycnidium. The spores in the parasitized host and on artificial media are oval, hyaline, continuous, and thin-walled; they measure 2.3–3.6 $\times$ 4.5–9.0 μ (average 2.6 $\times$ 6.2 μ).

Cicinnobolus was readily isolated: single pycnidia were picked off of the leaf surface with a needle and transferred to artificial media. The colony characteristics when grown on artificial media were similar to those described by Emmons.[1] Restricted growth de-

[1] Emmons, Chester W. 1930. *Cicinnobolus cesatii*, a study in host-parasite relationships. Bull. Torrey Botan. Club, 57: 421-441.

Fig. 1. Leaf symptoms of powdery mildew on London plane, showing **A)** severe infection of a mature leaf, and **B)** stunting and distortion of the terminal growth.

veloped on 8 commercially or laboratory-prepared agar media. These media were potato dextrose, corn meal, asparagine, oatmeal, nutrient agar, malt extract, bran agar, and Czapek's. The greatest amount of radial growth (15 mm) occurred on laboratory-prepared PDA after 3 weeks of incubation at 24°C. On agar, growth was compact and predominantly submerged with no free aerial hyphae and was pale yellow in color. Numerous reddish brown pycnidia were formed after 2 weeks of incubation on PDA.—Section of Applied Botany and Plant Pathology, Illinois Natural History Survey, Urbana, Illinois.

A CONTROLLED-TEMPERATURE CHAMBER [1]

David L. Gerwitz, T. W. Sudia, and Richard D. Durbin[2]

There has been need for a small, versatile, and economical, controlled-temperature chamber for biological research and teaching. Such a chamber has been built of clear Plexiglass sheets, 3/16 in. thick. This material can be worked with ordinary shop tools and bonded watertight at room temperature with ethylene dichloride or a commercial plastic solvent. The chamber temperature is regulated by manipulating the ratio of hot to cold water passing through a jacket surrounding the actual chamber. Such a chamber can be built at a fraction of the cost of a comparable commercially made unit.

The basic construction is an inner box surrounded on 5 sides by a larger box. Over-all dimensions will suit the individual's need. The space between the 2 boxes, 2 in. in our unit, becomes the water jacket. The sixth side, the top, is so sealed that the inner box is exposed, permitting unrestricted access to the chamber. Building from the inside out is recommended to allow maximum access to the surfaces being bonded, which is essential if the box is to be watertight. The surfaces to be bonded are softened conveniently by placing them in aluminum-foil troughs partially filled with the solvent. The water inlet is placed at the bottom, extending 4–5 in. into the jacket. Several outlets are used to prevent possible pressure buildups within the jacket; they are bonded flush with the jacket at the extreme top. The inner box is supported on the bottom with 3/4-in. rod, and the space between the 2 boxes at the top is sealed with plastic. A single-layered lid is kept in place with grease. If desired, tubes may be bonded through the lid and the bottom of the jacket to provide inlet and outlet systems for feeding, watering, maintaining controlled atmospheric conditions, or conducting $C^{14}O_2$ feeding experiments.

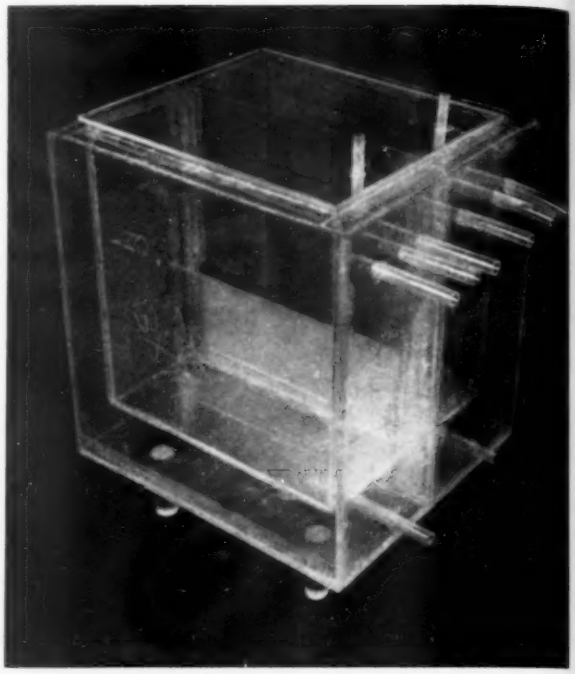

Fig. 1. A controlled-temperature chamber of Plexiglass. The divided inner chamber allows direct comparison of a specimen at different controlled temperatures.

The chamber can be used for growing microorganisms or, if lighting is provided, plants under controlled temperatures. A light intensity of 1500 ft-c midway between the top and bottom of the inner chamber of our unit is achieved by a bank of 8 cool while fluorescent and 3 75-watt incandescent lamps suspended 5 in. over the top. A fan provides air circulation between the lights and the unit. Water does not condense on the underside of the lid because of a 3°C temperature rise in the top 2 in. of the chamber. This differential is attributable to the lights; however, the temperature in the remainder of the chamber is constant within ±1°C so long as the flow rate and/or the water temperatures remain constant. If it is necessary to maintain different parts of a plant at different temperatures, the chamber may be subdivided into 2 sub-units, as illustrated in Fig. 1. The inner box is then supported by a double walled U-shaped saddle that divides the jacket into 2 independent units, each at a different temperature. The inner box is divided by a wall, the top portion of which is removable. A uniform temperature difference of 20°C has been maintained to within 1/4 in. of this dividing wall.—Department of Plant Pathology and Botany, Institute of Agriculture, University of Minnesota, St. Paul.

[1] Paper No. 4101, Scientific Journal Series, Minnesota Agricultural Experiment Station.

[2] Research Assistant, Research Fellow, and Assistant Professor, respectively, Department of Plant Pathology and Botany, University of Minnesota.

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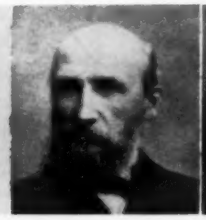

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NEWS

Editor: K. W. KREITLOW, Plant Industry Station, Beltsville, Md.

Phytopathologist Stephen Diachun has been appointed Director of the University of Kentucky gifted-student program. Dr. Diachun will devote half of his time to the new position working with the school's Honors Program Committee to bring more gifted students into higher education, and to arrange a curriculum which would permit them to work to the best of their abilities.

A lectureship in honor of J. C. Walker to be known as "The John Charles Walker Lectures in Plant Pathology" was recently established at the University of Wisconsin. The Chamber of Commerce and other friends of Dr. Walker gave several thousand dollars to the Wisconsin Alumni Research Foundation to endow the lectureship in the Department of Plant Pathology. The earnings from invested funds will be made available to bring eminent scientists to the campus.

Plant Pathologists Frederick L. Wellman, formerly In Charge, Coffee Research Project, Agricultural Experiment Station, Rio Piedras, Puerto Rico was named Head, Department of Botany and Plant Pathology, University of Puerto Rico. The new Department is a combination of two older groups; physiology and plant pathology.

An Oak Wilt Meeting was held in Bedford, Pennsylvania, November 12-13. Reports were heard from Pennsylvania, West Virginia, Maryland, Virginia, Kentucky, Tennessee and North Carolina. The Federal Survey and Control Activity was presented and oak decline problems in the Eastern States were discussed by State and Federal plant pathologists.

Plant Pathologist W. P. Skoropad assumed duties as Assistant Professor of Plant Pathology, in the Department of Plant Science of the University of Alberta, Edmonton, Alberta on September 1. Dr. Skoropad had been on the staff of the Plant Pathology Laboratory, Canada Department of Agriculture at Edmonton before joining the University.

Cercospora leaf spot of tung will be studied by Tom van der Zwet at the U. S. Field Laboratory for Tung Investigations, Bogalusa, La. Dr. Zwet recently received the Ph.D. degree in Plant Pathology from the Louisiana State University.

A nematode symposium entitled "Nematology in the Tropical and Subtropical Zones of the Western Hemisphere" was held the morning of December 2 during the 19th Annual Meeting of the Soil and Crop Science Society of Florida at the University of Florida, Gainesville. Participants in the Symposium were G. Steiner of Puerto Rico, Ing. R. Cuellar of Panama, E. J. Wehunt of Honduras, and W. A. Feder, U. S. Department of Agriculture, Orlando, Florida.

Phytopathologist George W. Buchenau, who recently completed graduate study at Iowa State University, has joined the Plant Pathology staff of the South Dakota State College to investigate diseases of cereals.

A scholarship fund to be known as the Bonde Scholarship Fund has been established by members of the Department of Botany and Plant Pathology and other friends and associates of the late Dr. Reiner Bonde, University of Maine. The fund will be used for the education of Dr. Bonde's children. Contributions can be sent to Drs. Richard Campana, Merle Hilborn and Franklin Manzer. Fund collections will be terminated by July 1, 1960.

Phytopathologist Gene Easton was recently appointed Assistant Plant Pathologist, Maine Agricultural Experiment Station. Dr. Easton will conduct basic and applied research on diseases of potatoes and other vegetable crops with particular reference to virus problems.

The Potomac Division will hold its 17th annual meeting February 25-26 in the auditorium of the Plant Industry Station, Beltsville, Md.

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